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Prioritizing cover crops for improving root health and yield of vegetables in the Northeast
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Phytopathology 101:S1

Cover crops are used increasingly by growers to improve soil quality, prevent erosion, increase organic matter, and suppress root pathogens and pests. However, limited information is available on their use for suppressing pathogens (Rhizoctonia, Pythium, Fusarium, Thielaviopsis, Pratylenchus, and Meloidogyne) of vegetables grown in the Northeast. Thus, a collaborative project was initiated in 2009 to assess the efficacy of selected cover crops in suppressing root pathogens of vegetables and improving soil health in research and on-farm field trials in New York, Pennsylvania, and Connecticut. In NY, strips (4.5 x 60 M) of 9 cover crops (rye grain + hairy vetch, oat, sudex, forage radish, red clover, rapeseed, buckwheat, wheat, and a fallow check) were randomized in 4 fields with 3 replications (3.2 ha total). The fields had different management histories resulting in varied levels of pathogen pressure and soil quality. In 2010, cover crop biomass was measured and collected soil samples were assessed for root health (greenhouse bean bioassay), nematode diversity and density, and selected soil health parameters (Cornell Soil Health Test). In general, root rot severity was lowest and yield of snap bean was highest in the field with the highest soil quality. After one year, the cover crops greatly affected root health and bean yield in this trial as well as the microplots and/or on-farm trials conducted in CT and PA. Another cycle of evaluations is in progress.

Reduction of aflatoxins, cyclopiazonic acid and fumonisins in corn by biocontrol strains of non-aflatoxigenic Aspergillus flavus
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Phytopathology 101:S1

Non-aflatoxigenic biocontrol strains of Aspergillus flavus were examined for ability to reduce, production in corn of aflatoxins and cyclopiazonic acid (CPA) by A. flavus and fumonisins (FBs) by Fusarium verticillioides. The ability of non-aflatoxigenic strains to prevent aflatoxin production by subsequent challenge with toxigenic A. flavus strains was assessed in 4 experiments. Non-aflatoxigenic strain K49 effectively prevented toxin production at various inoculation levels in 3 experiments. K49 also was evaluated alongside the widely used biocontrol strains NRRL 21882 (Afla-Guard®) and AF36 for prevention of aflatoxin and CPA production by strains K54 and F3W4. K49 and NRRL 21882 were superior to AF36 in reducing aflatoxins. K49 and NRRL 21882 produced no CPA, and reduced CPA and aflatoxin production in a subsequent challenge with F3W4 and K54 by 84–97% and 83–98%, respectively. In contrast, AF36 inoculation and subsequent challenge with F3W4 reduced aflatoxins by 20% and 93% with K54, but showed no CPA reduction with F3W4 and only 62% CPA reduction with K54. Because AF36 produces CPA, high CPA accumulated in corn with AF36 alone. Pin-bar wounding and pin-bar inoculation with F. verticillioides NS-2 resulted in FBs levels of 253 and 1087 ppm, respectively. Inoculation with K49 alone or a mixture of K49 and NS-2 reduced FBs level to 0.1 and 27 ppm, respectively. AF36 and NRRL 21882 showed similar FBs reduction trends to K49. NRRL 21882 and K49 are effective in reducing aflatoxins, CPA and FBs in corn.

Managing potato scab and enhancing tuber yield with low rates of fish emulsion applied as a pre-plant soil amendment
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Phytopathology 101:S1

Fish emulsion (FE) is an excellent organic soil amendment to enrich soil microbes and generate disease suppressive conditions against soil-borne diseases such as seedling damping-off, potato scab, and verticillium wilt. However, the rates (20,000 L/ha) of FE that provided effective control of potato scab can be too costly for commercial use. The aim of this 3-year field study was to see if much lower rates of FE could suppress potato scab and increase tuber yield. Diluted FE (1000 and 2000 L/ha or 0.05 and 0.1%) was applied to the field plots twice a year before planting and after harvesting potatoes starting in fall of 2007. The high rate of FE (2000 L/ha) consistently reduced scab severity by 42% in 2008, 57% in 2009, and 44% in 2010; reduced the percentage of tubers with deep-pitted scab by 30% in 2008, 51% in 2009, and 66% in 2010; and increased the percentage of marketable tubers by 21% in 2008, 55% in 2009, and 12% in 2010. Both rates of FE increased total tuber yield by 16–19% in 2008, 14–20% in 2009, and 7–11% in 2010. FE soil amendment enhanced the numbers of soil bacteria including those of potential bio-control agents belonging to the genera Pseudomonas and Bacillus. These results suggest that economically feasible rates of FE applied more frequently can provide disease suppression and enhance tuber yield. Next step is to monitor the lasting impact of these disease suppressive conditions on continuous potatoes without any further FE application.
Effect of arbuscular mycorrhizae on aphid infestation of wheat

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Phytopathology 101:S2

In greenhouse-grown pots of wheat serendipitously infested with bird cherry oat aphid, *Rhopalosiphum padi*, the aphids preferred non-mycorrhizal treatments over treatments with arbuscular mycorrhizal (AM) fungi. Therefore, studies were developed to determine if mycorrhizal infection reduced the ability of wheat to support a population of *R. padi*. Two AM fungi *Glomus intraradices* (Gi), *Gigaspora margarita* (Gm)) were propagated on sorghum, and non-mycorrhizal sorghum served as a control. After three months, vegetative growth of sorghum was removed, and cultures, both with and without AM, were mixed 1:1 with growing medium. Wheat (*Triticum aestivum* L.) was sown directly onto the adopted sorghum. The plants were grown in the greenhouse for three weeks. Twenty apterous aphids were transferred to each plant. Three pots/treatment were used in Trial 1; six pots/treatment were used in Trial 2. After 5 days, aphids were counted on each plant. Mycorrhizal treatment had no impact on germination and survival of wheat plants. Number of aphids/plant was approximately 2.5 times greater on non-mycorrhizal plants than on Gi- and Gm- colonized plants [Trial 1 (P = 0.0912); Trial 2 (P = 0.0955)]; number of aphids on colonized plants were intermediate and not different from the other treatments.

**Multiplex PCR for four Sclerotinia species**

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Phytopathology 101:S2

*Sclerotinia* homeocarpa, *S. minor*, *S. sclerotiorum*, and *S. trifoliorum* are common species within the genus *Sclerotinia*, where the morphological identification is challenging, especially when one crop hosts multiple species. The objective of this study was to design species specific primers compatible with multiplexing, for rapid and accurate species identification. Highly specific primers working under similar PCR conditions were designed for the asparagine synthetase (*asnS*), the *S. sclerotiorum*, the elongation factor-1 alpha gene of *S. homeocarpa*, and the laccase 2 gene of *S. minor*. The specificity and sensitivity of primers were tested individually and in multiplex against isolates of each species and each consistently amplified DNA of their target species only. Four DNA fragments of different sizes were amplified: a 264-bp PCR product for *S. minor*, a 218-bp product for *S. homeocarpa*, a 171-bp product for *S. sclerotiorum*, and a 97-bp product for *S. trifoliorum*. Primer sets differed in their lower sensitivity limits: SMlac2 = 1 pg/µL; SHelf1 = 0.1 pg/µL; SSaspr, and STCad = 10 pg/µL. Primer sets were used in Trial 1; six pots/treatment were used in Trial 2. After 5 days, aphids were counted on each plant. Mycorrhizal treatment had no impact on germination and survival of wheat plants. Number of aphids/plant was approximately 2.5 times greater on non-mycorrhizal plants than on Gi- and Gm- colonized plants [Trial 1 (P = 0.0912); Trial 2 (P = 0.0955)]; number of aphids on colonized plants were intermediate and not different from the other treatments.

**Sensitivity of tomato early blight isolates** (*Alternaria solani*) from Jordan to mancozeb, chlorothalonil and azoxystrobin fungicides

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Phytopathology 101:S2

The in vitro sensitivity of tomato early blight pathogen (*Alternaria solani*) isolates from Jordan were evaluated to the fungicides mancozeb, chlorothalonil, and azoxystrobin. Sensitivity of sixty single conidium isolates of *A. solani* to mancozeb and chlorothalonil was assessed using the inhibition of radial mycelial growth (RG) method, using fungicide concentrations of 0, 1.0, 10, 100, 500, and 1000 µg a.i. ml⁻¹ medium. The sensitivity of each isolate to azoxystrobin was measured by assessing the conidial germination inhibition of each isolate on water agar plates amended with 0.0, 0.001, 0.01, 0.1, 1.0, and 10 µg/µL. The EC50 value (concentration of the fungicide causing 50% relative reduction of radial mycelia growth or spore germination) values of different *A. solani* isolates to mancozeb ranged from 9.05 µg/ml to 712.65 µg/ml, and had a mean of 187.12 µg/ml. EC50 values of different isolates to chlorothalonil ranged from 4.25 µg/µL to 849.4 µg/ml and had a mean of 153.65 µg/ml. Forty three isolates, and thirty isolates of *A. solani* exhibited reduced sensitivity to mancozeb and chlorothalonil respectively, with EC50 values more than 100 µg/ml. The EC50 values of different isolates to azoxystrobin ranged from 0.040 µg/ml to 1.09 µg/ml and had a mean of 0.29 µg/ml. Forty six isolates of *A. solani* exhibited reduced sensitivity to azoxystrobin with EC50 values more than 0.1 µg/ml.

**Comparison of endophytic *Undifilum* DNA and swainsonine content on locoweeds**

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Phytopathology 101:S2

Locoweeds are toxic species of *Astragalus* and *Oxytropis*, associated with the endophytic fungus *Undifilum oxytropis*. The fungus produces swainsonine, an α-mannosidase-inhibiting alkaloid which causes cell damage to mammals and plants. We used real-time PCR to quantify *Undifilum* DNA (UD) from field samples and in vitro grown plants. Amplification of the ribosomal ITS allowed reliable quantification of UD in plant tissues. UD in *in vitro* *O. sericea* increased between the first and third week of growth, whereas swainsonine concentration started increasing between the second and fourth week. A strong correlation existed between the amount of UD and the swainsonine content for *O. sericea* grown on a culture medium during the first four weeks. Addition of polyethylene glycol to the medium significantly impaired the plant development, accelerated endophyte colonization (40 pg UD/ng total DNA at Week 2), and increased the swainsonine concentration. Acidification of the medium resulted in increased plant growth and minimal swainsonine content. UD ranges were estimated in locoweeds populations in New Mexico and Colorado and annual cycles were described for swainsonine content. Both UD and swainsonine content coincided at their lowest, while swainsonine concentration variable when medium or high amounts of endophyte DNA were found. Our findings highlight the reliability of qPCR for studying endophyte colonization and show environmental cues affecting the swainsonine synthesis in planta.

**Kasugamycin in combination with copper or mancozeb for management of walnut blight in California**

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Phytopathology 101:S2

Walnut blight caused by *Xanthomonas arboricola pv. juglandis* (*Xaj*) is one of the most economically important diseases of walnut in California and worldwide. Copper resistance has developed in pathogen populations and is widespread throughout CA. Screening of new bactericides has led to the identification and development of kasugamycin for management of fire blight and other bacterial diseases. Baseline sensitivity studies were conducted to determine the range of toxicity to *Xaj* and to aid in monitoring antibiotic sensitivities in field populations. *Xaj* exhibited a relatively high and continuous range of sensitivities to kasugamycin (LC 36 to 44 µg/ml; MIC 44 to 70 µg/ml) as compared to other bacterial plant pathogens such as *Erwinia* and *Pseudomonas* spp. In 2008, 2009, and 2010, kasugamycin significantly reduced blight incidence by 50% to 75% as compared to the untreated control. In combination with copper or mancozeb, however, the efficacy of the antibiotic was significantly improved and incidence was reduced by more than 85% in orchards with copper-resistant pathogen populations. In orchards with copper resistance, the efficacy of the antibiotic was higher when used alone or in the combinations. Thus, kasugamycin in combination with copper or mancozeb and in rotation with copper-mancozeb mixtures should reduce the selection for resistance to the antibiotic and reduce the overall usage of mancozeb and copper as the only highly effective treatments available.

**The filamentous phage phiRSS1 enhances virulence of *Ralstonia solanacearum***

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Phytopathology 101:S2

The RSS1 is a filamentous phage infecting *R. solanacearum*, the causative agent of bacterial wilt. This phage was frequently integrated in the host genome. This study was conducted to understand the effects of phiRSS1 infection on *R. solanacearum* cells in their interaction with host plant. Upon infection, phiRSS1 affects the physiological state, pathogenicity, and behavior of the host cells. The major changes include 3.53 fold increase of extracellular polysaccharides, increase (28.46%) of endoglucanase activity and 1.46 times faster of twitching motility compared with uninfected cells. These changes also affected the virulence and pathogenicity. Tomato plants inoculated with phiRSS1-infected bacteria killed 2 to 3 days earlier than those with uninfected bacteria. These results clearly showed that phiRSS1 infection enhances host virulence.

**Foamy bark rot of Fukumoto navel: A condition with etiology not yet understood**

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Phytopathology 101:S2
Fukumoto navel orange, which was introduced into California in 1983 has many desirable attributes but foamy bark rot condition is a serious concern for the variety. Symptoms include splitting of the bark of the trunk and branches that leads to the release of gum exudates and at times production of whitish foamy substance that smells like a beer brewery. Samples were collected from infected Fukumoto trees in Tulare, Fresno, and Kern counties, where the variety is commonly grown in California. Screening was done using morphological and molecular detection methods for Phytophthora spp., fungi, and bacteria. Some of the frequently isolated organisms include Phytophthora citrophthora, four bacterial genera (Bacillus spp., Pantoea sp., Pseudomonas spp. and Acinetobacter), and several fungi (Alternaria citri, Botryosphaeria spp., Fusarium solani, F. oxysporum, and F. equiseti). Although probing was done using PCR and specific (C. cucurbitae) - specific primers, none has been found so far. Pathogenicity tests are being conducted with Fukumoto trees propagated with disease-tested budwood onto Carrizo and Volkameriana rootstocks supplied by the California Citrus Clonal Protection Program. Preliminary data indicate that a combination of two or more of these organisms may play a role in the foamy bark rot condition.

**First report of Tomato mosaic virus on eggplant in Iran**

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Phytopathology 101:S3

During the June 2009 to August 2010, symptoms of leaves mosaic, interveinal necrosis and fruits discoloration were observed in eggplant fields in Golestan Province, Iran. A total number of 20 leaf samples collected from symptomatic eggplants and tested for the presence of Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), Arabis mosaic virus (ArMV) and Cucumber mosaic virus (CMV) by DAS-ELISA using commercial specific antibodies (Agdia, Elkhart, IN, U.S.A.) according to the manufacturer’s instructions. Among the samples, ToMV was detected in 55% of the samples. ArMV and TMV were not detected in any of the tested samples. Total RNA was extracted from each sample and analysed by RT-PCR using a pair of primers that flank the coat protein gene (CP) of Tomato mosaic virus (ToMV). The 750-bp ToMV-specific DNA fragment was amplified in all tomato samples collected from 3 fields in Karaj district and all mechanically inoculated tobacco plants. Ampliments were not produced from healthy plants or the water used as negative controls. RT-PCR products were purified and directly sequenced. BLAST analysis of ToMV (GenBank Accession No. HQ593624) sequence showed 98% nucleotide identity with reference sequences (AJ492083, AJ492086, AF012917, AF378152, AF067233, AF067231) deposited in the NCBI database. The experimental results confirm the observed symptoms were caused by ToMV in the eggplant in Gorgan region. ToMV has previously been reported from Iran, but this is the first report of ToMV in eggplant in Iran.

**Cross-infection of Colletotrichum species on tropical fruit in postharvest**

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Phytopathology 101:S3

The genus Colletotrichum is considered one of the major fungal pathogens of plants, that pathogen is distributed worldwide and affects different crops before harvest and postharvest. In the present study were isolated and identified morphologically and molecularly eight isolates of Colletotrichum sp., isolated of banana, papaya, starfruit, chile manzano, avocado, mango manila and mango ataulfo. These were inoculated on each of the fruits to assess the phenomenon of cross-infection. Diameter of injury was measured daily for seven days. The results showed that some isolates of C. gloeosporioides are more aggressive when inoculated on their original host, but cause less damage in alternate hosts, and other isolates of this species have more aggressive when inoculated in alternate hosts than on their original host. On the other hand C. musae having a smaller number of hosts. The fruits of starfruit were resistant to different isolates of C. gloeosporioides and C. musae. The fruits showed higher susceptibility were, banana and papaya, as they were affected by all isolates.

**Red palm weevil, Rhynchophorus ferrugineus (Oliver)**, the worst invasive pest of palms

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Phytopathology 101:S3

Red Palm Weevil (RPW), Rhynchophorus ferrugineus (Oliver) has been identified by the FAO of the United Nations as a ‘category-1’ insect pest of date palm, Phoenix dactylifera, and currently 18 other palms are reported as hosts of this pest. RPW was first described as a serious pest of the coconut palm, Cocos nucifera, in 1906, while in 1917 it was described as a serious pest of the date palm in the Punjab, India. In the mid of 1980’s, it is invaded the Gulf Countries of the Middle-Eastern region from where it moved into Africa (Egypt) in the early 1990’s and subsequently into Europe (Spain) by transporting of infested offshoots. Lately, it is pest of Phoenix canariensis in the Mediterranean basin. In 2005, 2006, 2007 RPW is reported from Greece, Turkey, Canary Islands, Cyprus, France, Italy, Portugal and Malta. While in 2008, 2009 and 2010, it is reported from Morocco, Albania, Republic of Georgia (Sukhumi). From Curaçao Island/Netherland Antilles, Libya, Lebanon and U.S.A. Where it arrived through infested palms. RPW mostly infests young palms less than 20 years old with a single female laying about 300 eggs, which hatch into the most damage stage. All stages (egg, larva, pupa and adult) are inside the palm itself. Early infestation of RPW is difficult to detect. RPW is currently managed through a pheromone based Integrated Pest Management (IPM) strategy. Field sanitation and cultural practices are one of the important components to prevent weevil infestation. No effective biological agent has been found.

**Using the tomato spotted wilt virus nucleocapsid protein gene for pathogen-derived resistance in lettuce**

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Phytopathology 101:S3

Tomato spotted wilt virus (TSWV) is the most important viral disease affecting Hawaii lettuce growers. Earlier work by others showed that the transgenic introduction of the TSWV nucleocapsid protein (N) gene into lettuce can confer pathogen-derived protection on those plants. In this work, four constructs were created using the N-gene in various expression contexts and inserted into lettuce cv. Grand Rapids via Agrobacterium-mediated transformation. At maturity, the primary transformatants exhibited poor seed set which may have been due to environmental conditions. However, R0 plants exhibited good seed set with segregation ratios indicating the presence of a single transgene. R1 plants were challenged with TSWV obtained from field-collected infected tomato. From this, it is expected to express the entire N gene in plus-sense showed good resistance. Twenty-four transgenic plants were challenged. At ten days post inoculation, six plants showed good resistance with no lesions, seven showed moderate resistance as measured by lesion number and nine showed limited or no resistance. Non-transgenic control plants were showing symptoms of systemic infection; numerous and confluent lesions were found on the inoculated leaves, and lesions as well as some vein necrosis appeared on non-inoculated, systemically infected leaves. Inoculation challenges of the remaining transgene constructs in lettuce are underway, and this set of transgene constructs is also being used for the transformation of Romaine lettuce.

**Relationships between nematode distribution in pine stem and development of xylem embolism observed with a compact MRI in pine wilt disease**

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Phytopathology 101:S3

Pine wilt disease, caused by pine wood nematode (Bursaphelenchus xylophilus) introduced from North America, is one of the most serious tree diseases in the north temperate zone. This disease is known to induce xylem embolism caused by xylem occlusion and feeding activity of the nematode in infected pines. Previous studies have shown that a number of nematodes grow around the infection site at the early stage, and then start rapidly moving and multiplying through the tree at the later stages of this disease symptom. It was unclear whether the distribution of nematode corresponds to the development of xylem embolism. In this study, development of xylem embolism was monitored nondestructively and 3-dimensionally with multi-cross-sectional slices taken by a compact MRI in Japanese black pines and compared to the distribution of the nematodes with staining nematodes with F-WGA. In a set of transgene constructs is also being used for the transformation of Japanese black pine.

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Phytopathology 101:S3**
Identification of small molecule inhibitors against SecA of Candidatus Liberibacter asiaticus by molecular modeling studies

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Phytopathology 101:S4

Plant pathogenic bacteria are responsible for overwhelming losses in citrus agriculture. Huanglongbing (HLB) is one of the most devastating diseases of citrus crop in many countries. Candidatus Liberibacter asiaticus (Ca. L. asiaticus) is the causal agent of HLB disease. Treating Ca. L. asiaticus infected citrus trees is one attractive goal due to the great value of citrus trees and the high cost of citrus tree removal and replanting. Identification of small molecule compounds which inhibit the activity Ca. L. asiaticus is an alternate approach to control the HLB disease. SecA is an ATPase translocate enzyme of Ca. L. asiaticus which involves in pre-protein translocation between the membrane and ribosome membrane. Interrupting the ATP-hydrolysis process of SecA by small molecule inhibitor would disrupt the pre-protein translocation and this could lead to the possible antimicrobial compound. In the current study, homology modeling, structure based virtual screening & molecular docking studies have been used to identify the small molecule inhibitors. Finally we found twenty compounds at micro to nano molar activity against SecA. Optimization of the high activity compounds will lead to potential antimicrobial candidates that could be used to control the HLB disease. All the molecular modeling studies have been performed on Linux system by using Maestro module of Schrodinger suite softwares. ATPase assay kit and purified SecA enzyme was used for biological testing.

Detection of Grapevine leafroll-associated virus 7 using Real-time® qRT-PCR and conventional RT-PCR

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Phytopathology 101:S4

Grapevine leafroll-associated virus 7 (GLRaV-7) is an unassigned member in the Clusteroviridae family that was first recorded in an asymptomatic white-berried grapevine cultivar from Albania. In California, the virus has been detected in several cultivars including Chardonnay, Merlot, Pinot Noir, Emperor, Black Seedless and Sauvignon Blanc. An ELISA test is available for GLRaV-7, but that assay has been described as unsuitable for field use. In order to improve field detection of this virus, sequences in the coat protein gene (CP) and the heat shock protein homolog gene (hHSP70) from nine Californian GLRaV-7 isolates were compared. Consensus sequences derived from those comparisons were used to design both RT-PCR primers and qRT-PCR assays. When 77 grapevine samples were screened with these two detection methods, the qRT-PCR assay based on the hHSP70 sequence identified more positives (13.86%) than the one based on the CP sequence (9.24%). Both qRT-PCR assays (CP and hHSP70) appeared to be more sensitive than the RT-PCR assay which detected only 2.31%.

Grapevine leafroll-associated virus 1 occurs as genetically diverse populations in grape wine cultivars

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Phytopathology 101:S4

We studied the genetic diversity of thirty-four field isolates of Grapevine leafroll-associated virus 1 (GLRaV-1) from different wine grape cultivars in California, New York and Washington States in the U.S. Genetic segments of the heat-shock protein-70 homolog (HSP70h), coat protein (CP), coat protein duplicate 2 (CPd2) and ORF9 (p24) were amplified by RT-PCR, cloned and sequenced. A pairwise comparison of nucleotide sequences revealed intra- and inter-isolate sequence diversity, with CPd2 being the most diverse among the four gene segments. An estimation of dN/dS values indicated different selective pressures acting on each of the four genomic regions. A global phylogenetic analysis of the HSP70h and p24 gene sequences revealed segregation of GLRaV-1 isolates into three major lineages with isolates from the U.S. distributed in all three lineages, indicating a lack of clustering by geographical origin. Such a segregation of GLRaV-1 isolates into three lineages was not apparent when the CP and CPd2 gene sequences were used for phylogenetic analysis. The molecular incongruence in phylogenetic trees suggests that the three lineages could represent different and unrelated putative recombination events among the four genomic regions further revealed a higher degree of natural variation in GLRaV-1. The genetic landscape of GLRaV-1 will provide a foundation for better understanding the epidemiology of grapevine leafroll disease across grape-growing regions in the U.S.

Impacts of grapevine leafroll disease on an own-rooted wine grape cultivar

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Phytopathology 101:S4

We conducted studies in an own-rooted Merlot block to measure impacts of grapevine leafroll disease (GLRD) on grapevine (Vitis vinifera) performance, fruit yield, berry and wine quality. For this purpose, grapevines were identified in a commercial vineyard in such a way that individual grapevines exhibiting typical GLRD symptoms and tested positive for Grapevine leafroll-associated virus 3 were adjacent to healthy grapevines in a given row to minimize error in sampling and experimental results due to variations in growing conditions. Data collected during 2010 season showed differences in leaf photosynthesis and chlorophyll fluorescence between GLRD-affected and unaffected leaves only after veraison. Fruit maturity indices (soluble solids and fruit acidity) and total anthocyanins measured at various stages of berry development showed significant differences between berries produced by GLRD-affected and unaffected grapevines. Fruit yield measured at the time of commercial harvest showed significant reduction in GLRD-affected grapevines. Small-plot wines made from grapes harvested from GLRD-affected Merlot grapevines showed significantly less amounts of pigments (anthocyanins, small- and large-polymeric pigments), tannins and alcohol than in wine made from fruit harvested from unaffected grapevines. These results demonstrate that GLRD affects vine performance and impacts fruit and wine quality in own-rooted Merlot grapevines grown under cool-climate conditions.

Determination of presumptive vegetative compatibility groups of Verticillium dahliae occurring on sunflower using molecular markers

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Phytopathology 101:S4

Verticillium wilt on sunflower is caused by Verticillium dahliae, and was managed by the dominant gene V1 for the last two decades. A new strain in North America (NA-Vd2) is able to overcome the V1 resistance gene. In Argentina, where Verticillium wilt is endemic, a strain different from that in North America is hypothesized. However, the VCGs of V. dahliae sunflower isolates have never been characterized from any country. Thus, characterizing sunflower V. dahliae VCGs, knowing their relative aggressiveness, and studying their genetic diversity are important in breeding programs and in screening the resistant lines efficiently. A total of 900 V. dahliae isolates were collected from sunflower and other hosts from the U.S., Canada, Australia and Argentina. DNA was extracted from all V. dahliae isolates and six polymerase chain reactions and AFLP analysis were used to study population variability. Five PCR patterns have been found and are designed A, B, C, or D. In some instances there were no amplifications (NA). PCR patterns varied based on host and geographic origin. Preliminary results of North American isolates showed that most V. dahliae isolates recovered from sunflower belong to VCG-2A.

Profile of Pythium spp. in certified organic fields for vegetable production in central Washington

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Phytopathology 101:S4

Damping-off is a common disease of many crops, including vegetables. The disease is exacerbated in organic production by the lack of highly effective organic seed treatments. Pythium is the major pathogen typically associated with damping-off in early season plantings in temperate regions, when soil and irrigation water are cold. The objective of this study was to identify Pythium species associated with damping-off in organic vegetable production in the semi-arid Columbia Basin of central Washington, particularly in pea and sweet corn crops. In October 2009, soil samples from 37 certified organic fields with a history of pea and/or sweet corn production were analyzed for a species associated with damping-off in early season plantings in organic vegetable production. Pythium spp. were baited from the soils using grass leaves and root baiting methods with damping-off in early season plantings in organic vegetable production. Pythium spp. were baited from the soils using grass leaves and root baiting methods with damping-off in early season plantings in organic vegetable production.
dissotocum, P. irregularare, P. sylvaticum, P. ultimum, and P. violae) caused damping-off, with differences in aggressiveness detected among isolates of each species. Inoculum levels of these species in the sampled soils will be quantified using real-time PCR assays.

Aggressiveness of Sclerotinia sclerotiorum from the north central United States on multiple crops

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Phytopathology 101:S5

Sclerotinia sclerotiorum is a pathogen of many crops yet little is known about aggressiveness within a diverse population from multiple crops. A collection of isolates from the North Central states were evaluated for aggressiveness as measured by lesion length. Thirty isolates from six mycelial compatibility groups (MCG) with five isolates in each MCG were evaluated on six crops: drybean, canola, lentil, pea, soybean and sunflower. Another 67 isolates, representing 31 MCG’s, were evaluated only on dry bean and sunflower. Plants were inoculated 5 to 7 weeks after planting using the cut-stem technique, placing a straw or pipette tip with agar and mycelium over the cut end of the stem, then misting plants for three days. In the experiments with the 30 isolates, all isolates were pathogenic and crop was a significant factor with the longest lesions on dry bean and the shortest on pea. However, MCG, isolate within MCG, and the interactions of crop x MCG, and crop x MCG x isolate were not significant factors. All of the 67 isolates were pathogenic on both crops and isolate was a significant factor, but crop was not. There were significant differences within an MCG among isolates in aggressiveness, suggesting that MCG may not be related to pathogenicity factors.

The role of rice rhizobacteria in defense against Magnaporthe oryzae infection

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Phytopathology 101:S5

Rice blast disease, caused by the fungal pathogen Magnaporthe oryzae, infects all foliar parts of the rice plant. Exploitation of the natural alliance between soil microbes and rice roots can increase protection against rice blast through induction of systemic resistance (ISR). ISR is mediated through intra-plant signaling and can be triggered by direct bacterial root colonization of plant growth promoting bacteria or through microbial volatile components. Bacteria isolated from California field grown M104 (temperate japonica variety) were tested against M. oryzae strain 70-15 in vitro and in planta. The volatile components of a Pseudomonas isolate, EA105, indirectly inhibited 70-15 growth. Five days post co-inoculation, the fungal diameter averaged 55 percent of the control. To see if this inhibition was due to hydrogen cyanide production, a known cyanide producer P. fluorescens CHAO was tested. CHAO averaged 59 percent of the control diameter, yet exhibited more aerial hyphae than EA105, indicating a better living environment for 70-15 compared to an EA105 volatile exposed one. Gas chromatography-mass spectrometry method will be used to determine the volatile components. EA105 root treated rice plants were not harmed by the inoculum and priming of roots with pentaeryrithrol for 24 hours failed to reduce blast lesion formation when infected with 70-15 spores. However, additional biological replicates must be performed to confirm this ISR-like response to pathogen infection.

Incidence of Fig leaf mottle-associated virus and Fig mosaic virus in Eastern Province of Saudi Arabia

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Phytopathology 101:S5

Fig plant, Ficus carica L., is grown in Saudi Arabia and is being affected by fig plant mosaic diseases (Fig leaf mottle-associated virus, Fig mosaic virus). The main symptoms are chlorotic mottling, blotching and various types of leaf deformation. Samples were collected, with consideration of the economically importance and distribution of the cultivation, from different areas of the East Province of Saudi Arabia. Each sample was consisted of 10-15 leaves. Samples were mixed into a mashing buffer and stored at −20°C till used. 8–10 µl of TNA extracts were mixed with 1 µl random hexamer primer, (Boehringer Mannheim, GmhH) (0.5 µg/µl). RT-PCR assay of leaves extracts of infected fig accession using specific primers gave positive results and non with FLMaV-2. Mixed infection of FLMaV-1 and FMV were found. To our knowledge this is the first record and identification of FLMaV-1 and FMV in Saudi Arabia. Further studies are needed to investigate the fig mosaic disease throughout the country.

Virulence variability and genetic diversity among Cochliobolus sativus isolates recovered from barley and wheat in North Dakota

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Phytopathology 101:S5

Cochliobolus sativus (anamorph: Bipolaris sorokiniana) causes economically important diseases including common root rot and foliar spot blotch in cereals. In North Dakota, the diseases occur in both barley and wheat which are concurrently grown in the region. However, the relationship between the pathogen populations recovered from the two crops is poorly understood in terms of virulence and host specificity. In this study, 30 C. sativus isolates recovered from barley and 30 C. sativus isolates recovered from wheat were inoculated on two-leaf-stage seedlings from three barley genotypes (Bowman, NDS883, and NDB112) and four wheat genotypes (Grandin, Chris, PI 644122, and PI411132) in the greenhouse. The results indicate that the majority of the wheat isolates were more virulent on the wheat genotypes than the barley isolates. In contrast, the barley isolates were more virulent on the barley genotypes as compared to the wheat isolates. However, variability in virulence was observed among the isolates derived from the same host. Amplified fragment length polymorphism (AFLP) analysis was performed on the 60 isolates tested for virulence and a high level of polymorphism was revealed using four primer combinations (E-ATM-CT, E-ACM-CC, E-AGM-CA, and E-AA-M-CA). The relationship between phenotypic variation and DNA polymorphism in the pathogen population will be presented.

Maintaining Maturity Group IV soybean seed quality: Perspectives from Mississippi, 2009 and 2010

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Phytopathology 101:S5

Numerous factors can reduce Maturity Group IV soybean seed quality. Over the past two years, research in Mississippi has attempted to determine strategies to reduce losses attributed to poor seed quality. Environmental conditions during the 2009 season resulted in severe quality losses that could be attributed to mold, stink bugs, and additional environmental factors influencing harvested seed quality. Foliar applications of the fungicide azoxystrobin (291 ml/ha of Quadris) and insecticide bifenthrin (380 ml/ha of Brigade) were made at 3 locations at R3, R5, and R5 + R5, alone and tank mixed. Locations sustained different environments resulting in mild (Stoneville), moderate (Raymond), and severe (Starkville) seed quality damage. Return-on-investments (ROI) for these treatments were calculated and while some applications did reduce observable percent mold there were still excessive ROI losses. In addition, secondary trials were conducted whereby azoxystrobin applications were made at numerous timings (R1, R3, R5, R6) and final concentrations (0, 291, 581, 876, 1168 ml/ha). At Raymond, number of applications ($R^2 = 0.4387; p = 0.025$) as well as final fungicide concentration ($R^2 = 0.5000; p = 0.004$) were significant factors. Additionally, the number of insecticide applications significantly reduced percent mold ($R^2 = 0.620; p = 0.003$). Optimal conditions during 2010, resulting in no seed quality losses, made assessing treatments and their overall effect on seed quality difficult.

Identification and characterization of promoter elements from plant pararetroviruses from dahlia (Dahlia variabilis)

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Phytopathology 101:S5

Dahlia mosaic virus (DMV), Dahlia common mosaic virus (DCMV) and an endogenous plant pararetrovirus (DMV-D10) are three distinct dahlia-associated caulimoviruses whose promoters have not been characterized. Based on sequence comparisons and promoter prediction programs, the promoters of these promoters from these three viruses was identified. The promoter regions were independently cloned into pCAMBIA1281Z. All constructs were delivered into Agrobacterium tumefaciens by electroporation, and agroinfiltrations were done into Nicotiana benthamiana. The activity of the 35S promoter homologs was determined by transient expression of the beta-glucuronidase gene (GUS). The length of the promoter regions corresponded to 438 bp for DMV, 439 bp for DCMV and 256 bp for DMV-D10. Quantitative GUS assays demonstrated that the promoter activity of 35S homologs from DMV and DCMV was similar to that of Cauliflower mosaic virus.
virus 35S promoter in *N. benthamiana* leaf tissue, whereas significantly lower promoter activity was observed for DMV-D10. Qualitative GUS assays were consistent with quantitative GUS results.

### Rapid immuno-test combined with magnetic bead technology on site-detection of potato leafroll virus

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Phytopathology 101:S6

The rapid on-site detection of plant pathogens is relevant for growers and inspectors. Lateral-flow tests for recognizing five of the six most important potato viruses are available, but the detection of potato leafroll virus (PLRV) has not been possible with the conventional lateral-flow format, presumably due to low virus titer. In order to overcome this shortcoming, the PLRV AgriStrip assay was developed. This lateral-flow test is based on antigen-antibody reaction combined with magnetic bead technology, enabling the preceding enrichment of target antigen (PLRV). Potato leaf extracts are first incubated with magnetic beads coated with antibodies specific for PLRV. The beads bind the viral antigen and are then separated from the extract with a magnet and resuspended in a small volume of running buffer. After inserting the strip into the enriched solution, the concentrated beads migrate upwards and colored test- and control-lines become visible, indicating the health status of test samples. The PLRV AgriStrip-magnetic assay employs mono- and polyclonal antibodies and reacts specifically with PLRV. No cross-reaction is observed with other potato viruses. The sensitivity attained with the AgriStrip-magnetic test is comparable to the sensitivity in the DAS-ELISA method. With the availability of the AgriStrip-magnetic, potato sample extracts can be analyzed in small volumes for the presence of the six most important potato viruses PVA, PVM, PVX, PVY and PLRV.

### Resistance of *Brachiaaria* genotypes to *Rhizoctonia* spp.

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Phytopathology 101:S6

Foliar blight, caused by *Rhizoctonia* spp., is an important production limitation to *Brachiaaria* pasture-based livestock in the Tropics. The potential for increasing genetic host-plant resistance to foliar blight will depend on the genetic variation for pathogenicity and virulence in the fungal populations, in the genetic variation in resistance in the host plant, and in the possible interaction between pathogen and host-plant variability. The present study sought to initiate characterization of pathogen variability encountered within Colombia and the interactions between this variation and relevant host-plant variability. We obtained 147 *Rhizoctonia* isolates collected from different *Brachiaaria* genotypes in five Departments of Colombia. Isolates were morphologically characterized and their pathogenicity was evaluated in artificially inoculated trials under greenhouse conditions. Isolates differed both in their morphology and their aggressiveness. Isolates from Meta, Casanare, Cordoba, and Caqueta were more pathogenic than those from Cauca. Restriction fragment length polymorphism analysis classified the isolates into two groups: *R. solani* anastomosis group AG-1 IA and *Rhizoctonia* spp. AG-D. A study of virulence showed that multicellular *Rhizoctonia* isolates had the highest virulence. Pathogen isolate, host genotype, and isolate-genotype interactions were all significant sources of variation (P > 0.0001).

### A new *Phytophthora* sp. causing basal rot on Japanese iris

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Phytopathology 101:S6

Japanese iris (*Iris ensata* var. *ensata*) is a popular flowering plant that is widely cultivated in Japan. Recently, a disease causing basal rot accompanying initial yellowing of a central leaf on the plants, has occurred in many iris gardens. These symptoms are visible from the early growing season until the flowering stage in wet cultivation conditions. Homothallic *Phytophthora* sp. was first isolated with high frequency from diseased plants collected at Suigou Sawara Aquatic Botanical Garden in Chiba prefecture, Japan. Typical symptoms developed on the plants when inoculated by root dipping in water containing cultured agar pieces of the fungus for 20–24 hr before planting to soil. The same fungus was recovered from the diseased tissues. The fungus formed oogonia with paragonous antheridia, oospores turning golden brown when aging, and non-papillate zoosporangia. Sequence analyses of rDNA-ITS region, beta-tubulin gene, and elongation factor 1 alpha gene revealed that the isolate showed similar homology with *Phytophthora europaea*. Its morphological and cultural characteristics (Jung et al., 2002) were almost coincident with those of the iris isolate. The isolate, however, were clearly distinct from *P. europaea* in phylogenetic trees. It was also found that the same disease caused by the clonal fungus was widely de-veloped in Japan. We concluded that *Phytophthora* sp. isolated from Japanese iris differs from other known species in genetic characters and host plants.

### Localization of *Candidatus Liberibacter* asiaticus associated with huanglongbing in various organs of its psyllid vector using FISH and Q-PCR

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Phytopathology 101:S6

*Candidatus Liberibacter* asiaticus (Las) has been associated with huanglongbing (HLB), or citrus greening, which is currently the most devastating citrus disease worldwide. HLB is transmitted by the Asian citrus psyllid *Diaphorina citri* (Hemiptera, Psyllidae) in a persistent manner, but its vector interactions, particularly at the organ and cellular levels, are poorly understood. We used fluorescent *in situ* hybridization (FISH) and quantitative PCR (Q-PCR) for the localization of Las in *D. citri*. Las was detected by FISH in the hemolymph, filter chamber, midgut and salivary glands of HLB-infected *D. citri* and in the phloem of infected citrus leaves. Additionally, Q-PCR detected Las in dissected organs of individual *D. citri* adults collected from field-affected trees or a laboratory-infected colony. The proportion of Las-infected (PCR-positive) salivary glands (47–70%) was significantly lower than that in other body parts (79–98%). The relative titer of Las, compared to psyllid genomic DNA in each sample, was significantly higher in both the salivary gland and alimantary canal compared to that in the rest of the body. These results provide the first molecular localization of Las in the hemolymph, alimantary canal and salivary glands of *D. citri*. They also strongly suggest that the salivary glands constitute an important infection and/or transmission barrier to Las in the psyllid vector, and that Las may replicate or accumulate in both the alimantary canal and salivary glands of *D. citri*.

### A new detached-leaf assay to test the inoculativity of psyllids with *Candidatus Liberibacter* asiaticus associated with huanglongbing disease

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Phytopathology 101:S6

To test the inoculativity of the Asian citrus psyllid (ACP) *Diaphorina citri* with *Candidatus Liberibacter* asiaticus (Las), associated with huanglongbing (HLB) disease, we used a new detached-leaf assay in which ACP adults from a HLB-infected colony were fed for 1 week on detached healthy sweet orange leaves placed in 50-ml polypropylene tubes. One week later, these leaves were processed for quantitative PCR (Q-PCR) using two Las primers (Li and Lj900). When feeding 10, 5 or 1 ACP/leaf/week the percentages of Las-positive leaves were 40, 18.8 and 4.4%, respectively, using Li primers, and 60, 40.6 and 11.1%, respectively, using Lj900 primers. Ct values for Q-PCR using Lj900 primers were much lower than those using Li primers. Our results, using the more commonly used but less sensitive Li primers, are largely comparable to those obtained by previous workers using whole citrus seedlings for Las-inoculation by ACP. However, using more sensitive primers can increase the usefulness of this method. We suggest that this new ‘detached-leaf assay’ method can potentially speed up Las-inoculativity tests on ACP from 3–12 months to only 2–3 weeks, which can greatly enhance pathogen-vector relation studies on Las and ACP.

### Genetic diversity and DNA fingerprinting of *Xanthomonas oryzae pv. oryzae* isolates from east and central Africa


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Phytopathology 101:S6

Genetic diversity and DNA fingerprinting of *Xanthomonas oryzae pv oryzae* (Xoo) isolates from east and central Africa was carried out using a newly developed PCR technique that combines molecular diagnostic and DNA fingerprinting for Xoo identification and differentiation. Molecular PCR diagnostic showed that the presence of at least a band indicates positive detection of Xoo pathogen and absence of a band indicates negative for no Xoo pathogen detected, while in the same PCR assay the presence of one or more band at different position revealed the DNA fingerprint of each Xoo.
isolates. Molecular diagnostic revealed that all the 28 Xoo isolates from Uganda, Mozambique and Rwanda including control from IRRI Philippines were Xoo pathogens. DNA fingerprinting of the 28 Xoo isolates revealed two major Xoo genotypes (XooG1 and XooG2) in Uganda, Mozambique and Rwanda. Interestingly, XooG2 genotype is typical of Uganda origin only while XooG1 genotype constitutes Xoo isolates from Uganda, Mozambique and Rwanda. The study revealed Xoo pathogen population structure and evidence of Xoo pathogen migration and movement within the three countries which could be due to poor seed health gernplasm exchange across the region. Development of a reliable molecular technique for Xoo identification and differentiation is a prerequisite into understanding the genetics of Xoo population structure in east and central Africa and development of durable resistance cultivars. Key words: Xanthomonas oryzae pv. oryzae, Molecular diagnostic, DNA fingerprinting, PCR technique, Genotype, Africa.

Influences from long-term crop rotation, soil tillage and fertility on the severity of rice grain smuts

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Phytopathology 101:S7

False smut (Ustilaginoidea virens) and kernel smut (Neovossia hiorida) are diseases of rice (Oryza sativa) that reduce both grain yield and quality. Susceptible rice varieties are in widespread use on production acreage in the United States, and the effects from crop management practices on smut control are poorly understood. We studied the long-term effects of crop rotation, soil tillage and fertility level on rice-smut severity. The highest levels of false smut observed in this study were on varieties grown in rotation with soybeans, on traditionally tilled soils, with high fertilizer treatments. The highest levels of kernel smut were observed in a rice-soybean rotation with winter wheat grown between summer crops. These rotations are commonly used in rice growing regions of the southern U.S. Using combinations of crop rotation, soil tillage and fertility rate, several alternative crop management practices were identified that provided effective control of smuts in susceptible rice varieties. The most effective method for controlling both false smut and kernel smut was in three-year rotations of rice, soybeans and corn. Regardless of rotation order, or tillage and fertility treatments within the rotations, rotating out of rice for two years was the most effective approach for smut control.

Development of mtCOI PCR primers with 5′ AT-rich flaps for rapid identification of high consequence Bemisia tabaci

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Phytopathology 101:S7

Whiteflies (Hemiptera; Aleyrodidae) are globally distributed agricultural pests. The Bemisia tabaci (Gennadius) sibling species group is a cryptic species composed of morphologically identical but genetically distinct biotypes (well-characterized) and haplotypes. Biotypes differ with respect to host colonization, fecundity, virus transmission efficiency, pesticide resistance, and invasiveness. Rapid identification of B. tabaci variants is necessary to facilitate interventions that minimize the likelihood of exotic whitefly introduction. The sequences of mitochondrial cytochrome oxidase I (mtCOI) and COII of B. tabaci from different regions of the world were used to design PCR primers that are rich in 5′ AT-rich flaps for rapid and sensitive identification of the B biotype, an approach that can now be extended to other economically important haplotypes.

Custom transcription factors for manipulation of gene expression in Phytophthora infestans

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Phytopathology 101:S7

Improvements to the genetic tools available for manipulating genes in oomycetes would enhance studies of gene function in these organisms. To help accomplish this we are testing the effectiveness of custom-engineered transcription factors based on two classes of DNA binding proteins with predictable DNA-binding specificity: zinc finger proteins (ZFPs) and transcription activator–like effectors (TALEs). Both ZFPs and TALEs have modular DNA-binding regions, each conferring recognition of one or three DNA base pairs respectively. Combining modules allows the assembly of DNA-binding domains with predictable binding specificity. In a variety of transgenic organisms (plants, yeast and mammalian cells), such custom ZFPs and TALEs fused to activation domains activate transcription of endogenous genes, whereas fusions to repression domains inhibit transcription. ZFP and TALE DNA-binding domains were designed to target sequences in the native INF1 promoter as well as to a minimal promoter (NilS) fused to a GUS transgene. Each of the DNA binding domains will be fused to a SIDs transcriptional repressor as well as to a VP16 activation domain, transformed into protoplasts of P. infestans, and the resulting transformants will be assayed for the expression of the TALE-SID/VP16 chimera, ZF-SID chimera, INF1, and TALE-GUS. Additionally, experiments will be performed with ZFPs and TALEs fused to the nuclease domain of FokI, which will be designed to target mutations to the INF1 coding sequences.

Systemic infection of coffee plants (Coffeea arabica L.) by Tobacco mosaic virus

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Phytopathology 101:S7

Tobacco mosaic virus (TMV) has proven to be a versatile gene expression vector in many different Nicotiana species. In this study we investigated the adaptation of TMV to infect coffee (Coffeea arabica L.) systematically, to determine if it could be used as a genetic tool for further studies in coffee. Previous studies have shown that coffee is host for few viruses. Coffee is naturally infected by the nuclearhabdovirus Coffee ring spot virus in Brazil and Costa Rica, whereas inoculation of different viruses including TMV resulted in only local infections. We inoculated TMV strain U1 virions onto leaves of sixteen 3-month old and sixteen 5-month old C. arabica cv. Caturra plants. Although no clear symptoms were observed in coffee leaves, ELISA tests of samples from inoculated leaves showed that all 32 plants were positive for TMV infection at 14 dpi. We found that TMV accumulates in coffee to a concentration that is approximately 13% the level found in N. benthamiana at 7 dpi, and that its titer increased in coffee over the next two weeks. We also demonstrated that TMV in upper non inoculated coffee leaves was detected by ELISA, and visualized virions in both types of tissues by electron microscopy. Furthermore, TMV virions purified from coffee were still infective after two passages through coffee. We are currently evaluating the capacity of TMV to serve as an expression vector in coffee. Project Funded by the Colombian Ministry of Agriculture.

Abundance and diversity of fungal endophytic community in an Italian beech forest: Pyrosequencing vs isolation method

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Phytopathology 101:S7

Since the mid-1980s, a decline of beech (Fagus sylvatica), one of the most important forest tree species in Italy, has been reported with symptoms consisting on general suffering of trees and tree death. Some endophytic fungi as B. nummularia, are known to lead to the onset of symptoms that arise with favourable ecological or physiological conditions. In this study the diversity of the fungal endophytic community in ten trees samples from an Italian beech forest has been assessed by cultivation method and high-throughput tag-encoded FLX amplicon pyrosequencing. A total of 149 cultures were isolated and identified on the base of morphological and molecular analysis. Pyrosequencing was carried out on amplicons derived from the fungal 18S rDNA region. A total of 31,676 reads were obtained. Trough phylogenetic assignment using BLASTN, taxa were identified. The values of abundance and diversity of fungi in the samples are linked to the method of detection used. A few fungal taxa account for most of the species’ abundance, whereas the majority of species are only rarely retrieved. Pyrosequencing resulted to be a reliable technique for investigating fungal communities in forest ecosystem.

Antimicrobial activity of essential oils of various plants against brown blotch disease on Agaricus bisporus

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Phytopathology 101:S7
Antimicrobial properties of essential oils of Mentha Piperita, Eucalyptus camaldulensis, Thymus vulgaris, Artemisia dracunculus were evaluated against Pseudomonas tolaasi, under in vitro condition. Extraction of EO from leaves were made with elevero. Bioassays for inhibition activities of EOs in three concentrations of $10^{-1}$, $10^{-2}$ and pure were performed against $P.$ tolaasi at $10^5$ cfu/ml on two agar media of NA and KB. After one day of incubation at $27^\circ$C, pure essential oil of Eucalyptus with $17$ mm inhibition zone, and pure EOs of Mentha and Tarragon with $2$ mm inhibition zone on KB medium exhibited the most and least antibacterial activities. No inhibition zone was observed for $10^{-2}$ concentration of Mentha, Thyme and Tarragon on NA medium. These were more pronounced when compared to least inhibitory effects of antibiotics erythromycin, penicillin and gentamicin in both concentrations of $10^{-1}$ and $0.01$ mg/ml. Tetracycline was an exceptional with similar result obtained from pure Eucalyptus EO. On the other hand, gentamicin and tetracyclin were more effective when the concentrations were increased into $1$, $5$, $10$ mg/ml.

Spray drift from aerial application on sugarcane in Brazil


The aim of this study was to develop and evaluate a method to quantify drift from aerial application on a sugarcane field. A 120 ha sugarcane field was sprayed with a solution containing rhodamine tracer. The application was done with an EMB 202 aircraft at 30 L ha$^{-1}$ with medium droplets, flight height from 2 to 3 m and 185 km h$^{-1}$. In order to evaluate the total drift by mass balance 20 horizontal glass collectors fixed on poles on the top of the canopy were randomly distributed on the field. Drift movement outside the field was evaluated by setting up a sampling line downwind where nylon string were placed at 10 m, 50 m, 200 m, 600 m, 1200 m and 2000 m from the field. The 2 m long strings were placed vertical on top of the canopy, with 8 replications for each distance. The spray application was done in 1.33 h, with meteorological averages of 19.6°C, 67.6% RH and 7.4 km h$^{-1}$ of wind speed. After the application the tracer was washed from the collectors and analyzed by HPLC. Based on this data a model to calculate the drift potential was developed. The total losses (drift) calculated by mass balance was 19.6%. Drift data downwind showed that a sugarcane plant growing 2000 m apart from the field would receive up to 3.9% of the dose rate applied on the field.

Performance of aerial application for soybean rust control and drift under unsuitable meteorological conditions for spraying


Phytopathology 101:S8

The aim of this study was to evaluate the drift potential and the performance of aerial applications for Asian Soybean Rust control under unsuitable meteorological conditions for spraying using a mixture of tebuconazol + picoxystrobin (0.5 + 0.25 L.c.p ha$^{-1}$) at a volume rate of 15 L ha$^{-1}$ and fine droplets. The five treatments were defined by the following adjuvants, all of them at 0.5% v/v: Oppa (mineral oil 930 g L$^{-1}$), Assist (mineral oil 756 g L$^{-1}$), Joint Mineral Oil (mineral oil 761 g L$^{-1}$), Oleo Vegetal Nortox (vegetal oil 930 g L$^{-1}$) e Vegetal Oil (vegetal oil 930 g L$^{-1}$). The 24 treatments were defined by the interaction of the six spray solutions with four variations of a 15 mm simulated rain: rain-free (no rainfall after application), 0 min (rainfall immediately after the application), 30 min. (rainfall 30 minutes after the application) and 60 min. (rainfall 60 minutes after the application). The results showed that the highest control rates were obtained with adjuvants. There was advantage on the use of adjuvants when there was no rain or the rain-free period after the application was at least 60 minutes. When the rain was simulated immediately after the herbicide application none of the adjuvants presented significant difference to the herbicide alone, while the Oppa and the Joint Oil induced a reduction on the weed control rate and the Oleo Vegetal Nortox was the only treatment that provided more than 80% control. When the simulated rain was applied 30 minutes after the herbicide application, the Joint Oil showed the best performance.

Role of seaweeds occurring at Karachi coast in suppressing the root diseases of cotton and chili

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Phytopathology 101:S8

Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of seaweed in crop production systems. In this study, Sargassum binderi, Rhizoclonium implexum, Stoechospermum marginatum, Spatoglossum variable, Melanothamnus afacuhsainii, Stoeckya indica and Solieria robusta occurring at Karachi coast were applied as soil amendment two weeks before sowing of cotton (Gossypium hirsutum L.) seeds in clay pots. Application of some seaweeds and topsin-M (fungicide) and carbofuran (nematicide) showed more or less similar suppressive effect on root rotting fungi Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani and root knot nematode Meloidogyne javanica on cotton. Seaweed also showed a positive effect on plant growth by enhancing fresh shoot weight and plant height. In field plot experiments, on cotton and chili (Capsicum annum L.) seaweeds showed similar suppressive effect on soilborne pathogens and improved plant growth. Spatoglossum variable and Melanothamnus afacuhsainii also reduced the root ball per plant in cotton. Due to increasing concern over the chemical pesticides and fertilizers, seaweed resources could be used for the production of organic fruits and vegetables.

Canyon live oak (Quercus chrysolepis) is susceptible to boll hole infection by Phytophthora ramorum

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Phytopathology 101:S8

Canyon live oak (Quercus chrysolepis) was originally shown to be susceptible to leaf and twig infection by Phytophthora ramorum (cause of Sudden Oak Death, SOD). Recently, mortality of large Q. chrysolepis was observed in a SOD-affected forest along with trunk symptoms indicative of late-stage P. ramorum infection. Symptomatic trees showed spatial correlation with California bay (Umbellularia californica), the primary source of P. ramorum inoculum in oak-bay forests. However, the pathogen was not recovered from cankers sampled through 2009. To determine the susceptibility of Q. chrysolepis to P. ramorum, we inoculated 12–20 cm diameter, 120 cm long, logs of disease-free Q. chrysolepis and Q. agrifolia trees with mycelial plugs of 7 P. ramorum isolates. Inoculated logs were enclosed in plastic bags and maintained at 20°C growth chamber. At 8 weeks, 90% of the inoculations had resulted in visible cankers with a mean canker size of 57 cm$^2$ in Q. chrysolepis and 228 cm$^2$ in Q. agrifolia. P. ramorum was recovered from nearly all canker margins. Field inoculations were conducted on 18 Q. chrysolepis trees in July 2010. Over the next three months, only four inoculated trees showed minimal visible trunk symptoms (i.e., bleeding). In December 2010, four inoculated trees were destructively sampled. All inoculations resulted in cankers similar in size and appearance to those observed with the logs; P. ramorum was recovered from canker margins in all cases.

Phytophthora ramorum’s trophic nature suggests that it cannot utilize dead leaf litter in aquatic systems

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Phytopathology 101:S8

Phytophthora ramorum, cause of Sudden Oak Death, is routinely isolated from waterways using leaf baits, but the nature of its presence in open waters...
is unknown. It has often been recovered from waterways with no known terrestrial infestation nearby. To test P. ramorum’s capacity to utilize plant litter as a substrate in aquatic systems, we simultaneously exposed freshly picked rhododendron leaves with those killed by drying or freezing in two infused streams. Baits were deployed monthly during peak pathogen activity from January to June in each of two years. P. ramorum was recovered from 62% of the only 6% and 2% of the frozen and dried leaves, respectively. To further characterize P. ramorum’s trophic capacity, we incubated fresh, frozen and dried leaves separately in cups of water inoculated with P. ramorum or P. gonapodyides (an ubiquitous, presumed saprobic resident of open waters), or combined inoculum of both species for 7 days at 16°C. When incubated alone both species colonized all three bait types; however, when isolated most frequently from fresh leaves, while P. gonapodyides was isolated more frequently from frozen and dry leaves. When incubated together, P. ramorum was isolated more frequently from fresh leaves than P. gonapodyides, while the opposite occurred with frozen and dry leaves. These results indicate that P. ramorum may be limited biologically and ecologically from colonizing litter in aquatic environments.

Strategies for management of southern corn rust in Georgia

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Phytopathology 101:S9

Southern rust of corn caused by *Puccinia polysora*, a disease of sporadic importance in Georgia. Objectives here included initiation of sentinel plots for early detection of southern rust and assessment of fungicide efficacy. Sixteen sentinel plots planted to southern rust-susceptible Pioneer 33M57 and rust-resistant Pioneer 33M52 were assessed in 2010. Earliest detection of southern rust was on 2 July (P33M57 Turner Co.); latest detection was on 29 July (P33M57 Mitchell Co. and P33M52 Turner Co.). Fungicide studies were conducted in three locations. Plot in Decatur Co. were planted to P33M52 and P33M57, to P31D59 in Tift Co., and P31D59 and DKC69-71 in Mitchell Co. Treatments in Decatur and Tift Counties included pyraclostrobin, pyraclostrobin + metconazole, azoxystrobin + propiconazole, tebuconazole, and fluoxastrobin or trifloxystrobin + propiconazole. Fungicides were applied at tassel or silking. Fungicides at P31D59 and DKC69 and P31D59 and P33M57 Mitchell Co. in Mitchell Co. (P33M57 Turner Co.).

Detection and discrimination of *Pythium aphanidermatum* and *P. delicu* by single probe based real time PCR and multiplex end point PCR

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Phytopathology 101:S9

*Pythium aphanidermatum* and *P. delicu* are closely related species and plant pathogens causing seed disease, damping-off, and root rot on many relevant crops growing in warm regions and greenhouses. The discrimination between these two *Pythium* species is difficult if based only on morphological characteristics. To speed the specific detection and discrimination of these two *Pythium* spp. two systems are presented: 1) A single probe Taqman real time PCR in which the probe (PAD) was designed to anneal with a conserved region located between two sets of primers specific for *P. aphanidermatum* (PA4Fa /PA4R) and *P. delicu* (PD1F/PD1R). These two sets of *Pythium* primers each amplify products of 143 bp. 2) A multiplex end point PCR that incorporates a new forward primer (PA4FB) located upstream of the conserved rDNA-ITS sequence. In this assay primer PA4FB and PA4R amplify a product of 346 bp that allows end point PCR discrimination of these two *Pythium* species. 5' A/T rich sequence extensions were added to all primers to enhance multiplexing efficiency. None of the tested primers amplifies DNA from six other *Pythium* species. All PCR products were cloned and sequenced to confirm identity. The described PCR assays are useful for detection, quantification, microbial forensic discrimination, biosecurity monitoring, and study of Oomycete ecology and biology.

Sensitive detection and discrimination of WSMV, TriMV and HPV using multiplex RT-PCR

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Phytopathology 101:S9

*Wheat streak mosaic virus* (WSMV), *High plains virus* (HPV) and *Tricomic mosaic virus* (TriMV) all damage cereal crops in Oklahoma, Kansas and the High Plains, and it may be difficult to determine whether symptomatic plants are infected with one or multiple virus species. A multiple RT-PCR assay that facilitates early, accurate and sensitive detection of WSMV, TriMV and HPV from plant tissues is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists to predict, identify and manage disease outbreaks and reservoir hosts. Specific PCR primers and probes were designed to target *Phytium aphanidermatum*. Sixteen sentinel plots planted to southern rust-susceptible Pioneer 33M57 and rust-resistant Pioneer 33M52 were assessed in 2010. Earliest detection of southern rust was on 2 July and 1% leaf area affected respectively; yields in 2010. Earliest detection of southern rust was on 2 July (P33M57 Turner Co.); latest detection was on 29 July (P33M57 Mitchell Co. and P33M52 Turner Co.). Fungicide studies were conducted in three locations. Plot in Decatur Co. were planted to P33M52 and P33M57, to P31D59 in Tift Co., and P31D59 and DKC69-71 in Mitchell Co. Treatments in Decatur and Tift Counties included pyraclostrobin, pyraclostrobin + metconazole, azoxystrobin + propiconazole, tebuconazole, and fluoxastrobin or trifloxystrobin + propiconazole. Fungicides were applied at tassel or silking. Fungicides at Mitchell Co. included pyraclostrobin and pyraclostrobin + metconazole applied at tassel or silking. Late season severity at Decatur and Tift Counties was less than 7% and 1% leaf area affected respectively; yields in plots treated with fungicides tended to be numerically but not significantly different from the untreated control. Late season severity at Mitchell Co. was less than 58% leaf area affected; yields in plots treated with fungicides were significantly greater than the untreated control.

Multi-gene based detection and identification of *Phymatotrichopsis omnivora*

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Phytopathology 101:S9

The soilborne fungus *Phymatotrichopsis omnivora* is the cause of cotton root rot and of rots of several dicots in the southwestern U.S. and Mexico. Diagnosis relies on characteristic mycelial cords, which are not always obvious, on the roots of wilted plants. A method facilitating early, accurate and sensitive detection of *P. omnivora* in plant tissues is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists to predict, identify and manage disease outbreaks and reservoir hosts. Specific PCR primers and probes were designed targeting *P. omnivora* genes for ITS-rDNA, beta-tubulin and the largest subunit of RNA polymerase II. Primer design was based on consensus sequences from multiple alignments. Three primer pairs and probes were validated in silico against published sequences and in vivo against infected plant samples. PCR products were cloned and sequenced to confirm identity. All primer sets allowed early detection of infected but non-wilted plants. Primer sets PO4 (116 bp product), P0B1 (126 bp product) and P0RBP2-2 (135 bp product) detected as little as 1 fg of plasmid DNA carrying *P. omnivora* target sequences at cycle threshold (Ct) values of 31.24, 30.54 and 30.9, respectively. The described PCR assays are useful for pathogen detection and disease diagnosis, microbial quantification, breeding programs, and biosecurity and microbial forensics.

Implication of phenezine-1-carboxylic acid production by *Pseudomonas sp.* LBUM223 in the biocontrol of *S. scabies* causing common scab of potato

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Phytopathology 101:S9

Experiments performed in our laboratory have revealed that *Pseudomonas sp.* LBUM223, which produces the antibiotic phenezine-1-carboxylic acid (PCA), is able to significantly alter the growth of *Streptomyces scabies*, the development of common scab of potato, and the production of thaxtomin A by the pathogen, which is required for pathogenesis. A mutant of LBUM223, producing PCA and producing PCA but incapable of producing thaxtomin A was recovered from these cultures. In order to better understand the impact of PCA production on the biocontrol of *S. scabies* under soil conditions, we characterized the population dynamics of LBUM223 (wild type and mutant) and the pathogen, as well the impact of PCA production on the expression of genes involved in thaxtomin A production in *S. scabies*. Potato plants were grown in pots and inoculated with different treatment combinations of *S. scabies* and LBUM223 (wild type and mutant) and harvested after 5, 10 and 15 weeks. Geocaulosphere, rhizosphere and bulk soil was collected and submitted to DNA and
Phytopathology 101:S10
(1) International Center for Agricultural Research in the Dry Areas
Rapid detection of formulations for field trials is under progress.

for plant protection against major fungal diseases. Development of hypal tip morphology and melanisation of hyphae. Also aborted and less within and across the groups of fungi. Morphological and physiological from 300ng/ml to 10 µg/ml. EV-050 was selective in inhibiting pathogens arachidis
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of glycerol/water. Embryos are cleared for easier examination. The new collaboration with Aleppo university, which reduced the test’s period detection method for barley loose smut was developed at ICARDA in ICARDA like most seed health and quarantine laboratories is detecting r to guaranty smut free seeds and reduce unnecessary uses of seed treatment. of this disease is highly likely in the field due to seed symptomless of this

infection is located inside the embryo. By exchanging seeds, the transmission of this disease is highly likely in the field due to seed symptomless of this

by PCA-producing LBUM223.

Current and future risk assessment of the spread of Trioza erytreae in citrus growing areas of North America
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Phytopathology 101:S10
Huanglongbing, caused by the phloem-limited bacteria Candidatus Liberibacter sp., is a devastating disease of citrus vectored by two psyllids. Within North America, the Asian citrus psyllid Diaphorina citri Kawayama is the only vector present. The threat of African citrus psyllid Trioza erytreae Del Guercio to North America remains a possibility. Previous work indicated that the African citrus psyllid is heat-intolerant, preferring temperatures less than the 30 degrees Celsius, while the effects of precipitation on distribution have not been studied. To determine if the climatic variables of temperature and precipitation will serve as a barrier to the vector, current distribution data on citrus and T. erytreae were gathered. Specifically the two variables of annual mean temperature and annual precipitation were examined for their effects on the psyllid distribution. Fine scale ecological assessment was performed using the maximum entropy algorithm MaxEnt with Akaake information criteria to determine which climatic variables are influencing the distribution. Additionally, two atmospheric-climatic general circulation models, CCCM and CSIRO, were used to predict the changes in suitable habitat in 2050. Precipitation is more strongly correlated to the psyllid distribution than temperature placing a higher risk for southern California and western Mexico for possible psyllid introduction than Florida.

Novel broad spectrum highly potent fungicide: EV-050
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Phytopathology 101:S10
A novel proprietary molecule EV-050 exhibited high potency and broad spectrum activity against fungi belonging to Ascomycota, Basidiomycota and Oomycota. Fungi that cause devastating diseases on economically important food crops, horticultural crops, ornamental plants and trees were tested against EV-050. In vitro efficacy of EV-050 against resistant strains isolated from field such as Botrytis cinerea, Alternaria solani, Coleotrichum gloeosporioides, Magnaporthe grisea was assessed by radial mycelial growth inhibition and conidial germination inhibition. IC50 varied from 900ng/ml to 55 µg/ml on selective pathogens. Disease index on infected leaf disc and spore germination was assessed for biotrophic pathogens such as Puccinia arachidis, P. viticola, Plasmopara viticola and Uncinula necator. IC50 varied from 300ng/ml to 10 µg/ml. EV-050 was selective in inhibiting pathogens within and across the groups of fungi. Morphological and physiological observations revealed that EV-050 targets virulence factors by altering the hypal tip morphology and melanisation of hyphae. Also aborted and less melanised appressoria led to impaired conidial germination. These results imply that EV-050 has potential to be developed as an antifungal agent for plant protection against major fungal diseases. Development of formulations for field trials is under progress.

Rapid detection of Ustilago nuda on barley (Hordeum vulgare)
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Phytopathology 101:S10
Loose smut of barley (Ustilago nuda) is strictly a seed-borne fungus where the infection is located inside the embryo. By exchanging seeds, the transmission of this disease is highly likely in the field due to seed symptomless of this pathogen; therefore increasing seed movement necessitates specific solutions to guaranty smut free seeds and reduce unnecessary uses of seed treatment. ICARDA like most seed health and quarantine laboratories is detecting r Ustilago nuda (7-013) method. This method is sensitive and reliable, but requires two days to complete the extraction. A new and fast detection method for barley loose smut was developed at ICARDA in collaboration with Aleppo university, which reduced the test’s period significantly to five hours only, where about 100 g seed is soaked in NaOH for 2 h; then heated for 30 sec at 70°C, the embryos are collected in 1 mesh; afterworlds embryos are separated using a solution of HCl, with mixture 1/1 of glycerol/water. Embryos are cleared for easier examination. The new method is fast, simple, reliable and very sensitive. This test can be used by seed health laboratories, regulatory and quarantine authorities to ensure that only loose smut seed free are introduced.

Analyses of nuclear and mitochondrial sequences reveal an ancient split in the evolutionary history of Verticillium dahliae
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Phytopathology 101:S10
Verticillium wilt, caused by the soilborne fungus Verticillium dahliae, is a ubiquitous disease of crops and ornamentals. In this study, the population structure of V. dahliae was examined using 16 microsatellite markers, eight nuclear exon and sequences of both nuclear and mitochondrial spacers. A total of 220 strains were collected from various hosts in four major agricultural valleys of California: Salinas, Santa Clara, San Joaquin and Sacramento. Migration analyses showed minimal gene flow among valleys. Interestingly, two major clades were consistently identified regardless of sequence targets. However, correlations of either clade with host and geographic origin, or virulence and vegetative compatibility phenotypes were undetectable. Using coalescent analyses on sequences of the eight nuclear exons, a molecular clock suggests that one clade split soon after divergence from a common ancestor. Conversely, the larger of the two clusters appears to be more recent. Strains from Europe and South America were placed in both clusters. These two clusters reflect a historical population structure of V. dahliae, which predates the pathogen migration into modern day California agricultural system.

Characterization of a single chemosensory gene cluster in Xylella fastidiosa Pierce's disease pathogen of grape
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Phytopathology 101:S10
Virulence of Xylella fastidiosa Temeculav involves the coordinate expression of a wide range of virulence factors including type IV pili which are associated with host colonization and twitching motility. Twitching motility in X. fastidiosa is regulated by a Pil-Chp chemosensory-like operon which is comprised of pilG, pilJ, pilK, pilL, pilP, chpB, and chpC genes and parallels the ChpIV chemosensory cluster found in Pseudomonas aeruginosa that regulates twitching motility and pili biogenesis. Mutations in pil-chp genes resulted in twitching-negative phenotypes and significantly reduced disease severity on grape cv. Cabernet Sauvignon, except with chpB and chpC. The mutants pilG, pilJ, pilK, pilL, and chpB produced less biofilm than wild-type whereas the chpC mutant did not. Furthermore, pilG, pilJ, and chpB mutants showed the least amount of aggregation followed by the chpC mutant. The pilL and pilJ mutants displayed the same level of aggregation as wild-type. Complemented mutants restored phenotypes to wild-type levels. These results indicate that this chemosensory system is also required for full virulence in X. fastidiosa.

Genetic diversity and population differentiation of Sclerotinia sclerotiorum collected from canola in China and in U.S.
Phytopathology 101:S10
Sclerotinia sclerotiorum is an important pathogen of canola and many other crops worldwide. Genetic diversity and population differentiation of S. sclerotiorum collected from canola fields in Anhui Province, China (30 isolates) and in North Dakota, U.S.A. (29 isolates) were investigated in terms of genetic variation in 8 simple sequence repeat (SSR) marker loci, mycelial compatibility groups (MCGs) and three phenotypic traits: sensitivity to fungicides benomyl, iprodione and fluzinam, oxalic acid production, and pathogenicity. Significant genetic differences were observed; there were no shared MCGs between the two populations. Population differentiation was significant (p = 0.0000) indicating lack of gene flow between the two populations. There were also significant differences between the two populations in oxalic acid production and in fungicide sensitivity. The Chinese population displayed high levels of insensitivity (faster growth rate) to benomyl and fluzinam and higher levels of oxalic acid production per unit dry weight of mycelium than did the U.S. population. However, there was no significant difference in pathogenicity between the two populations as measured by colonization of detached canola leaves. Data
suggest that despite geographic and genetic isolation the two populations of S. sclerotiorum were equally adapted to colonizing canola plants, and pathogenicity is under different selection pressure than the other genetic and phenotypic traits.

Role of soybean seed exudates in cultivar resistance to Pythium aphanidermatum

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Phytopathology 101:S11

Archer, a maturity group I cultivar with demonstrated flood tolerance and resistance to several Pythium spp., was compared to Hutcheson, a commercial group V cultivar, in response to the seed rot pathogen Pythium aphanidermatum. The purpose of this study was to identify and characterize seed exudates from two soybean cultivars presenting differential response to P. aphanidermatum seed rot. Dried seed composition was characterized by proximate analysis of moisture content, total phenolics, lipid content, and protein content. Isoflavones and carbohydrates identification and concentration were further determined for each cultivar. Hutcheson presented a higher concentration of carbohydrates, lipid content, and protein content, and a lower isoflavones concentration compared to Archer for both dried seed and seed exudates. An exchange exudates assay was conducted to determine the role of seed exudates to susceptibility to P. aphanidermatum. Hutcheson treated with Archer exudates presented a higher stand than Hutcheson control while Archer treated with Hutcheson exudates presented no differences in stand compared to Archer control. Likewise, a vegetative growth assay for P. aphanidermatum subjected to raw exudates showed differential diminution on radial growth with both cultivar exudates compared with the control. Overall, results suggest that seed exudates play a role in susceptibility to Pythium seed rot caused by P. aphanidermatum in soybean.

Development of a PCR-based assay for QoI resistance monitoring in the pecan scab pathogen, Fusicladium effusum

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Phytopathology 101:S11

Quinone outside inhibiting fungicides (QoIs) are an important component of integrated management programs for pecan scab, caused by Fusicladium effusum. The addition of salicylhydroxamic acid (SHAM) to fungicide-amended medium is toxic to F. effusum, rendering spore germination bioassays inadequate to quantify sensitivity of F. effusum to QoIs. An alternative molecular method was developed to assess QoI sensitivity in F. effusum, based on detection of amino acid substitutions G143A or F129L in the cyt bc1 gene sequences and all 77 isolates were found to be resistant to QoIs. A 530-bp cDNA fragment of the cyt bc1 gene was amplified from a wild-type isolate of F. effusum using a primer pair set-1 designed based on highly conserved regions of cyt bc1 gene sequences from several fungi, and sequenced. Based on the obtained sequence, a specific primer pair set-2 was designed to amplify the region containing the codons for G143A and F129L. DNA fragments from 77 F. effusum isolates collected from pecan orchards with a history of QoI use were amplified using this primer set and sequenced. Subsequent analysis revealed that all these cyt bc1 sequences and all 77 isolates were predicted to be sensitive to QoIs. This qualitative procedure will enable us to detect QoI resistance in F. effusum, should it occur, and subsequently monitor the frequency of QoI resistance relative to the use patterns of these chemistries.

Effects of downy and powdery mildew on juice grapes in Michigan

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Phytopathology 101:S11

Viticulture in Michigan is limited by a cool and humid climate and foliar diseases, such as powdery mildew (PM) and downy mildew (DM) that require frequent fungicide applications. In juice grapes (Vitis labrusca), PM and DM often appear after fruit set and their impact on vine physiology is still under study. To evaluate their effects, we established two disease levels (low and high) for both PM and DM in Niagara and Concord vines cropped at three levels (low, medium and high) by removing fruit clusters at 1200 growing-degree days (base 50 F, calculated from April 1). DM reached 53%, 57% and 73%, and PM <1%, 32% and 20% of the leaf area infected in 2008, 2009 and 2010, respectively. Fruit composition (Brix, pH and titratable acidity) was affected more by crop load than by disease, with the lowest Brix and pH values observed in diseased, high-cropped ‘Niagara’ vines. Cold hardness of ‘Niagara’ canes was significantly reduced by DM in 2008 and 2010 and by high crop load in 2009. Cane cold hardness was not significantly reduced in ‘Concord’. Bud cold hardness was unaffected in both cultivars. Starch content of the canes was reduced in both cultivars but only significantly in DM-infected ‘Niagara’ vines. At the levels of PM seen in ‘Concord’, fungicide applications did not appear to be cost-effective. However, ‘Niagara’ needs protection against DM to avoid a reduction in cold hardness, particularly under high cropping levels.

Responses of Rhizoctonia spp. and Sclerotium hydrophilum to the plant extracts

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Phytopathology 101:S11

Soilborne phytopathogens affect rice production by inhabiting inoculum permanently in the soil. Adverse effects of chemical compounds in disease control strategies cause a serious problem to the natural environment. Pesticides of plant origin are preferred in order to reduce the risks involved in chemical control measure. Sixteen naturally available phytoextracts were tested in vitro for their potential to control phytopathogens of rice such as Rhizoctonia spp. and Sclerotium. Among the tested phytoextracts, clove, neem leaves, rosemary and pelargonium are potential phytoextracts to control of the tested soilborne phytopathogens. All of the tested fungal growths were suppressed 100% by using clove extract. Neem leaf extract, rosemary extract and pelargonium extract were found to give the second best suppression against the tested fungi. Neem leaf extract inhibited the growth of R. solani by 87.5%, R. oryzae by 92.5% and R. oryzae-sativae by 80.0%. However, the same extract inhibited S. hydrophilum by only 49.1%. Rosemary extract gave an inhibition of 67.7% for R. solani, 88.0% for R. oryzae, 86.0% for R. oryzae-sativae and 73.8% for S. hydrophilum. The inhibitory effect of pelargonium on the tested fungi showed 48.1% for R. solani, 90.8% for R. oryzae, 84.4% for R. oryzae-sativae and 83.3% for S. hydrophilum. The present finding provided the information on the sources of phytoextracts to control rice sheath pathogens.

Identifying Macrophomina phaseolina genes involved in phytotoxin phaseolinone production using cDNA-AFLP analysis

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Phytopathology 101:S11

Macrophomina phaseolina is a soil and seed-borne pathogen that causes charcoal rot in soybean. Charcoal rot is one of the most destructive diseases of soybean in the United States. Little is known about the mechanisms governing the interaction between soybeans and M. phaseolina that result in charcoal rot development. The ability of M. phaseolina to cause charcoal rot may be associated with the production of the phytotoxin phaseolinone by the fungus. A cDNA-AFLP (Amplified Fragment Length Polymorphism) approach was used as a qualitative and quantitative tool to identify M. phaseolina genes involved in the production of phaseolinone. M. phaseolina was grown in conditions conducive and non-conducive to the production of the phytotoxin. The cDNA-AFLP screen was conducted using nine Mscl/EcoRI AFLP primer combinations. Sixty four unique transcript-derived fragments (TDFs) were found to be differentially expressed across the different growing conditions. The expression patterns of the corresponding genes were confirmed by quantitative Real Time PCR. This is the first report of M. phaseolina genes potentially involved in the biosynthesis of phaseolinone. These findings can be used to develop new tools to study the interaction between soybean and M. phaseolina.

A qPCR assay to detect and quantify Macrophomina phaseolina in soybean roots

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Phytopathology 101:S11

Macrophomina phaseolina is a soil and seed-borne fungal pathogen that causes charcoal rot. Charcoal rot can result in significant losses in soybean yields in affected fields. The use of soybean varieties resistant to M. phaseolina is one of the main approaches recommended to combat the disease. Screening for resistance to charcoal rot relies on assessing disease severity by scoring for root and stem symptoms. Resistance is also assessed by estimating the extent of fungal colonization of the roots and stem of the infected plant. This is usually achieved by determining the number of fungal colony forming units (CFU) obtained from the infected plants. In this study, we have developed a new quantitative polymerase chain reaction (qPCR) assay to assess the extent of colonization of infected soybean plants by M. phaseolina. Two Taqman probes were designed. The specificity and sensitivity of the probes were assessed and the correlation between colonization estimates of M. phaseolina obtained by qPCR, CFU counts and
field symptoms rating were assessed. A specific and sensitive qPCR assay would enhance the ability to screen for resistance to *M. phaseolina* in soybean.

**Bacterial spot** (*Xanthomonas cuscutae*): An emerging disease of pumpkin in Illinois  
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Phytopathology 101:S12

Bacterial spot, caused by *Xanthomonas cuscutae*, has become a serious threat to pumpkin production in Illinois. The pathogen infects foliage and fruit. Fruit infection by *X. cuscutae*, results in fruit rot. A study in 2009 showed that the disease occurred in all 17 commercial fields surveyed in Illinois. Another survey of 50 commercial fields in 2010 showed that the disease occurred in 80% of the fields, overall 34% of fruits with the bacterial spot symptoms. Incidence of fruit infection by *X. cuscutae* was greater than 50% in 18% of the fields, and the incidence of fruit infection exceeded 90% in 6% of the fields surveyed. During 2009–2010, 700 xanthomonad-like isolates were collected from infected pumpkin leaves and fruit using semi-selective medium [Kasznagycine-Cephalexin-Yeast extract- Peptone- Glucose-Agar (KC-Agar)]. Representative isolates (selected based on the colony morphology) were used in laboratory and greenhouse studies. Using genus-specific primers RST2 and RST3, xanthomonad isolates were selected. *X. cuscutae* was identified based on the biochemical and physiological characteristics. Pathogenicity tests of 19 *X. cuscutae* isolates were carried out on 3-week-old pumpkin (cultivar Howd en) plants by spray-inoculation of 20 ml of a 10^8 cells/ml water on the leaves in a greenhouse. Leaf spots developed 2 weeks after inoculation and *X. cuscutae* was isolated from inoculated leaves. Determining genetic variation among the isolates is underway.

A simple model for management of Fusarium crown rot in wheat  
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Phytopathology 101:S12

Crown rot caused by *Fusarium pseudograminearum* remains a persistent problem in wheat production despite decades of research. One possible reason for this is the lack of an accessible framework for understanding the effects of rotation, resistance and other practices on populations of the pathogen in the medium to long term. A simple descriptive model was developed for the disease in bread wheat in the northern grains region of Australia. Inoculum potential was estimated from the square root of the product of crown rot incidence and yield (a surrogate for biomass) of the preceding crop. Incidence of disease was related to inoculum potential by an infection constant. If wheat was not sown in the next season, crown rot inoculum declined exponentially with time. These two relationships were combined to predict the behavior of the disease in different rotation systems. Over time, crown rot incidence converges to the background level (7%) that was determined by the infection constant, average yield, and the decomposition rate of inoculum. Modeled epidemics behaved in a way that was consistent with field data for continuous wheat, wheat-chickpea and wheat-sorghum rotations. The model is not intended to give an accurate prediction of crown rot incidence under all conditions, but rather to allow generalizations about how the disease behaves. These can then be used in extension or as a way of understanding how management and environment affect the disease.

**Surfactin A isoforms characterizations in strains of Bacillus mojavensis for control of a maize pathogen, Fusarium verticillioides**  
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Phytopathology 101:S12

The endophytic bacterium *Bacillus mojavensis* has the potential for control of fungal diseases in maize and other plants. The bacterium and its cultural morphology) were used in laboratory and greenhouse studies. Using genus-specific primers RST2 and RST3, *Xanthomonas cucurbitae* isolates were selected. *X. cucurbitae* was identified based on the biochemical and physiological characteristics. Pathogenicity tests of 19 *X. cucurbitae* isolates were carried out on 3-week-old pumpkin (cultivar Howd en) plants by spray-inoculation of 20 ml of a 10^8 cells/ml water on the leaves in a greenhouse. Leaf spots developed 2 weeks after inoculation and *X. cucurbitae* was isolated from inoculated leaves. Determining genetic variation among the isolates is underway.

The results identified several strains as high producers of surfactin, as well as high producers of C-15 surfactin A, the most biologically active isoform for fungal toxicity, suggesting that it is these strains that should be used as biocontrol agents.

**Analysis of the Frankliniella occidentalis proteome and differentially expressed proteins in response to Tomato spotted wilt virus infection**  
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Phytopathology 101:S12

The western flower thrips (WFT), *Frankliniella occidentalis*, is an insect pest of agricultural importance and worldwide distribution. It causes direct and indirect damage by feeding on plants and transmitting tospoviruses, respectively. WFT is the most efficient vector of *Tomato spotted wilt virus* (TSWV), which replicates in both the plant host and the insect vector. The overall goal of this work was to develop proteomic tools to study this insect and its interaction with TSWV. Larval thrips were collected and their proteins separated by two-dimensional gel electrophoresis. Following staining, 194 protein spots were excised, trypsinized, and subjected to liquid chromatography with tandem mass spectrometers (LC-MS/MS). The mass-to-charge ratio as a function of time was then used to generate peptide sequences that were analyzed against the NCBI Metazoan database and a WFT expressed sequence tag (EST) collection. We found that 52% and 30% of the protein spots had significant matches to the WFT EST collection and the Metazoan database, respectively. We also used a proteomic approach to identify differentially expressed proteins between TSWV exposed and non-exposed larval thrips. We found that there are indeed changes in the thrips protein profile due to virus infection. Our findings provide new insight into the molecular basis of the interaction of WFT and TSWV. Ultimately, this knowledge will enable the development of novel ways to control thrips and tospoviruses.

**Genetic complementation between two viruses in an otherwise restrictive host**  
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Phytopathology 101:S12

Tospoviruses cause serious diseases in several important crop plants. The genome of tospoviruses consists of three RNAs, large (L), medium (M) and small (S). The L RNA is organized in negative sense orientation, whereas M and S RNAs are in ambisense. The S RNA codes for a non structural protein (NSs) in sense direction which was shown to function as viral suppressor of gene silencing in plants. We used datura (*Datura stramonium*) as a differential host for two distinct tospovirus species, Iris yellow spot virus (IYSV) and Tomato spotted wilt virus (TSWV). Following mechanical inoculation of datura, TSWV causes systemic infection, whereas IYSV infection of datura remains localized to inoculated leaves. We demonstrate that, in a mixed infection, the silencing suppressor NSs is expressed at a much higher level as compared to single infection in inoculated as well as systemic leaves. The systemic symptoms produced by TSWV in the presence of the IYSV silencing suppressor were more severe than those caused by TSWV infection alone. Even though the IYSV infection remained limited to the inoculated leaf, it was able to facilitate increased expression of TSWV NSs indicating complementation between two distinct tospovirus species.

**Biological characterization of distinct strains of Iris yellow spot virus (genus Tospovirus)**  
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Phytopathology 101:S12

Thrips-transmitted Iris yellow spot virus (IYSV) is an important limiting factor to the production of bulb and seed crops. Several IYSV isolates were identified from field samples in their putative host, *Datura stramonium* (a local lesion host) and *Nicotiana benthamiana* (a systemic host) following mechanical inoculation. Seedlings of both experimental hosts at four to six leaf-stages were mechanically inoculated. Host response was evaluated based on the following scorable phenotypic parameters: appearance of symptoms on inoculated leaves days post inoculation (DPI), DPI for the appearance of systemic symptoms on younger, un inoculated leaves, severity of symptoms, and effect on plant growth and vigor. Based on these parameters, two distinct strains of IYSV were identified. The duration in DPI that was necessary to
produce systemic symptoms and the subsequent death of inoculated plants varied between the mild and severe strains. In the case of the severe strain, systemic symptoms appeared 12 to 15 DPI and by 22 DPI, plants were severely infected and newly emerging leaves showed severe necrotic spots. By 50 DPI, inoculated plants died. The mild strain produced more benign symptoms as inoculated plants retained the vigor and optimal growth even after 60 DPI, with fewer new leaves showing systemic infection and the newly emerging leaves lacked symptoms.

Quantitative detection of Verticillium longisporum and V. dahliae in the soil of cabbage fields using nested-real-time PCR

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Phytopathology 101:S13

Verticillium longisporum and V. dahliae, causal agents of Verticillium wilt, are spread in cabbage fields of Gunma Prefecture. Based on the V. longisporum-specific intron within the 18S rDNA and also difference of ITS 5.8S rDNA sequence between F. longisporum and V. dahliae in Japanese isolates, we developed 3 types of the quantitative nested real-time (QNRT) PCR assays; V1-18S assay specific for V. longisporum, V1-Va-5.8S assay specific for V. longisporum and V. albo-atrum, and Vd-5.8S assay specific for V. dahliae. Quantification of V. longisporum or V. dahliae in cabbage field soil using the QNRT-PCR assays was well consistent with the disease severity of Verticillium wilt in those fields. We also carried out the field trials to estimate the effect of the cultivation of a resistant cultivar on pathogen population in soil. When resistant cultivar VR Raposo was planted for 3 seasons, both cabbage disease severity and pathogen density in the soil were significantly reduced in the field moderately contaminated by V. dahliae, but only slightly reduced in the highly contaminated field. In an additional field trial, initial pathogen density was correlated with Verticillium wilt disease severity. QNRT-PCR assays we developed here could be used to monitor the Verticillium pathogen population in field soils, and is a useful tool to assess the risk of Verticillium wilt prior to cabbage planting, to determine susceptibility to Verticillium wilt in cabbage cultivars.

How many species cause common and dwarf bunt of wheat?

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Phytopathology 101:S13

The wheat bunt pathogens are recognized as Tilletia caries, T. laevis (common bunt), and T. controversa (dwarf bunt). Characters used to delimit these species, including host symptomatology, teliospore morphology and germination, are continuous and accurate identification based on single isolates is not possible. Previous studies showing that the three species are reproductively compatible and possibly conspecific were based on a limited number of isolates that may not reflect the diversity present within this group of fungi. We tested the hypothesis that bunt pathotypes represent recently diverged species, and that monophyletic lineages can be identified among a geographically diverse set of isolates. Multilocus phylogenetic analyses based on three anonymous loci and a portion of RPB2 using 60 isolates from North America, Eurasia and Australia revealed two major clades but neither corresponded to recognized species. However, there was evidence of incongruence between the fungal taxa and nematode and nematode growth pattern suggestive of recombination. Hybridization, incomplete lineage sorting, and limited genetic variation, all of which are expected among recently diverged groups, complicate our ability to delimit species within these fungi. Different approaches are required for estimating species trees including the application of stochastic models to accommodate lineage sorting and to identify species in a way that is robust to both sampling and method.

The major fungal diseases of ornamental plants in Kerman Province, Iran

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Phytopathology 101:S13

Ornamental plants play an important role in interior scapes of our homes, offices, and streets. Ornamental plants in Kerman Province are produced under a wide range of environmental and cultural conditions and are affected by numerous pathogenic agents. Plant pathogenic fungi are the most important causal agents of ornamental plant diseases. To identify the fungal pathogens causing ornamental plants decline in Kerman Province, during 2010–2011 numerous samples were collected from ornamental plants (Tagetes spp., Petunia hybridra, Rosmarinus officinalis, Poo pratensis, Mattiola sp.) which were showing yellows, chlorosis and root and crown rot and declining symptoms. Several fungi were isolated from root, crown and stem of declining plants. The results showed that the prevalent species were Phytophthora cactorum on Mattiola sp., Phytophthora palmivora on Petunia hybridra, Rhizoctonia solani on Rosmarinus officinalis and Tagetes spp., Fusarium solani on Poo pratensis. The Koch’s postulation for each isolate were carried out and disease incidence were compared. Among fungal species recovered Phytophthora cactorum and Rhizoctonia solani were more pathogenic than the others and incidence of root and crown rot were observed more than the other disease symptoms.

Effect of immersion depth, dwell time and fruit-water temperature differences on water uptake by flamed tomatoes

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Phytopathology 101:S13

To prevent an internalization of decay and human pathogens into tomato fruit, packinghouses are instructed to limit the dwell time of fruit (<2 min), to warm the water at least 5°C higher than incoming fruit temperatures, and to limit the immersion of fruit to 1 ft (31 cm). The impact of these requirements on water uptake by fruit and the potential for subsequent decay development is unclear. Fruit immersed at 30.2 cm were exposed to a hydrostatic pressure of 29.6 mbar. Fruit that cool from 35 to 25°C are exposed to a final pressure differential of 33.1 mbar. However, this temperature decrease requires a dwell time >5 min. In comparisons of immersion depth versus fruit-water temperature differences (fruit warmer than water) with ≤2 min dwell time, depth was much more important to water uptake than up to a 20°C difference in temperatures. This is consistent with the percentage change in pressures on a cooling fruit, where a change in degrees Celsius + 273 affects the gas pressure inside versus outside the fruit. When suspensions of Erwinia carotovora were added to the water and the fruit dwell time was ≤2 min, the subsequent incidence and severity of bacterial soft rot were affected more by immersion depth than initial fruit temperature. Thus, with the current handling rules, water is likely to penetrate wounds on tomatoes exposed to a combination of the maximum depth of immersion and longest dwell time, whereas fruit-water temperature differences have little influence.

Relevance of the deposit structure for the biological efficacy of glyphosate as evaluated on four weed species

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Phytopathology 101:S13

The deposit pattern of foliar-applied agrochemicals, and its relation to the biological efficacy, has a big practical importance, but from the scientific point-of-view it is less understood. Thus, in our experiments we evaluated the relevance of the deposit properties for the bio-efficacy of hydrophilic a.i. - glyphosate. Deposition patterns were influenced by combining unformulated glyphosate with one selected ethoxylated rapeseed oil (RSO) surfactant having 30 or 60 ethoxylate (degrees Celsius + 273) units. Treatment solutions were applied to the foliage of easy-to-wet (Viola arvensis and SELLARIA media) and difficult-to-wet (Setaria Viridis and Chenopodium album) weed species. Deposit structure was determined using scanning electron microscope with energy dispersive X-ray microanalysis. Adjuvant-aided bio-efficacy of glyphosate was observed on all weed species. Surfactants with higher EO units reduced both a.i. and droplet spread area, and enhanced glyphosate toxicity in difficult-to-wet species. In general, on these plants, increasing EO unit led to shrinking of a.i. and droplet spread area which could have explained the observed bio-efficacy. In comparison of immersion depth versus fruit-water temperature differences, both cabbages and tomato seedlings were more pathogenic than tomato fruit and the potential for subsequent decay development is unclear. Fruit immersed at 30.2 cm were exposed to a hydrostatic pressure of 29.6 mbar. Fruit that cool from 35 to 25°C are exposed to a final pressure differential of 33.1 mbar. However, this temperature decrease requires a dwell time >5 min. In comparisons of immersion depth versus fruit-water temperature differences (fruit warmer than water) with ≤2 min dwell time, depth was much more important to water uptake than up to a 20°C difference in temperatures. This is consistent with the percentage change in pressures on a cooling fruit, where a change in degrees Celsius + 273 affects the gas pressure inside versus outside the fruit. When suspensions of Erwinia carotovora were added to the water and the fruit dwell time was ≤2 min, the subsequent incidence and severity of bacterial soft rot were affected more by immersion depth than initial fruit temperature. Thus, with the current handling rules, water is likely to penetrate wounds on tomatoes exposed to a combination of the maximum depth of immersion and longest dwell time, whereas fruit-water temperature differences have little influence.

Characterization of Tomato necrotic spot virus (ToNSV), a new Ilarvirus species infecting processing tomatoes in the Central Valley of California

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Phytopathology 101:S13

In 2008, virus-like symptoms including necrotic spots and streaks were observed in processing tomatoes in the Central Valley of California. Although these symptoms were similar to those induced by the ilarvirus Tobacco streak virus, tests for this and other known tomato-infecting viruses were negative. Results of sap- and graft-transmission experiments and sequence analyses of the capsid protein and replicase genes indicated that this disease was caused by a new Ilarvirus species, provisionally named Tomato necrotic spot virus (ToNSV). ToNSV is most closely related to Partitiviridae motte virus, another ilarivirus that infects tomato in Europe. Although ToNSV symptoms were common in processing tomatoes in the
Central Valley in 2008, the incidence was sporadic and relatively low (<1%) in most production areas in 2009. However, in 2010, ToNSV symptoms appeared early in the season and at higher incidences (up to 20% in some fields), and this was associated with high thrips populations. Additionally, the virus was detected in symptomatic field-collected peppers and onions. The results of ongoing studies on characterization and detection of ToNSV will be presented. This will include development of a rapid PCR-based detection method and studies on the mode of transmission, susceptibility of other crops and weeds, and the role of other hosts in the epidemiology of this new disease of processing tomatoes.

New species of the toxic fungal endophyte, Undifilum, from western United States locoweeds

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Phytopathology 101:S14

For over 100 years, locoweeds, from the genera Astragalus and Oxytropis, have been problematic for ranchers in the western United States due to their association with swainsonine toxin producing fungal endophytes. First described as Undifilum oxytropis from Oxytropis locoweeds, we present two new species of Undifilum. Taxonomic placement of fungi isolated from A. mollissimus and A. lentiginosus was based on growth rate, morphology, and molecular analyses. Isolates from A. lentiginosus grew more on alfalfa and potato carrot media than U. oxytropis. Astragalus mollissimus isolates grew only on potato dextrose media. Undifilum oxytropis were dark black with hardened outer surfaces, whereas isolates from Astragalus species did not exhibit the hard surface and were unique in coloring (A. mollissimus gray to black and A. lentiginosus tan to brown). Only fungi isolated from A. lentiginosus produced spores and these were similar to U. oxytropis with a slightly lower average number of septa. Maximum parsimony analyses of the ITS region and the gpd gene produced three clades (one for Undifilum oxytropis and one each for isolates from A. mollissimus and A. lentiginosus) and one distinct clade (A. lentiginosus isolates), resp. Neighbor-joining analyses of RAPD banding patterns showed one clade for U. oxytropis and one for isolates from Astragalus species. The results show fungi that are similar to U. oxytropis but have distinct features corresponding to new species within the genus.

Population genetics of Eutypa lata in the major grape-growing regions of the world and historical patterns of viticulture

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Phytopathology 101:S14

The causal agent of Eutypa dieback of grape, Eutypa lata (Ascomycota), is a destructive disease worldwide. The pathogen has a broad host range, but causes severe symptoms on only a few cultivated hosts (e.g., apricot & grape). To decipher its cosmopolitan distribution, we examined the population genetic structure of 19 geographic samples from grape in four continental regions (Australasia, Africa, Europe, and North America). Among loci supported the importance of sexual reproduction in all regions. The highest allelic richness \( H = 3.9 \) and gene diversity \( D = 0.001 \) were detected in a clade-specific manner from diseased field samples and 68–103 probes were 100% accurate for Undifilum oxytropis and one for isolates from Astragalus species. The results show fungi that are similar to Undifilum oxytropis but have distinct features corresponding to new species within the genus.

Effect of fungicide seed treatments and cultivars on Pythium damping-off and root rot of edamame soybean

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Phytopathology 101:S14

Production of edamame soybeans is increasing in Ohio, but Pythium damping-off and root rot can reduce productivity. The efficacy of fungicide seed treatments and cultivars against Pythium spp. was tested in 2009 and 2010. Seedling emergence and severity of Pythium root rot were assessed 3 and 10 weeks after seeding, respectively. Pythium colonies were recovered from root samples on PIBNC medium. Plant biomass was determined based on heights of plants from the center 5 ft of each row. Seed treatment with Apron XL+Maxim 4 FS+Cruiser (w or w/o Rhizobium inoculant) increased emergence and reduced root rot severity and the number of Pythium colonies compared to the untreated control in both years. Plant biomass was higher in plots treated with Apron XL+Maxim 4 FS+Cruiser than in the untreated control in 2010. Percent emergence was higher in the cultivar BeSweet 2015 than in BeSweet 2001 and BeSweet 292 in 2009. However, emergence was higher in BeSweet 292 than the other two cultivars in 2010. BeSweet 2015 had lower root rot severity than BeSweet 2001 and more Pythium colonies but higher biomass than the other two cultivars in 2010. Both cultivar and fungicide treatment should be considered to improve edamame emergence and root health in Pythium-prone soils.

Identification and evaluation of apple scab in Vf-resistant apple cultivars

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Phytopathology 101:S14

Apple scab, caused by the fungus Venturia inaequalis (Cke.) Wint., is one of the most important diseases of apples in the world. Up to 15 fungicide applications per year may be required in the eastern United States. An alternative approach for disease management is the use of Vf-resistant scab resistance from Malus floribunda 821. Vf-resistant cultivars were regarded as reliably resistant until 1993, when Parisi et al. reported the development of a new race of scab, capable of infecting the Vf-resistant cultivar, ‘Prima’, in Europe. Due to the process of introgressing the Vf gene into different genetic backgrounds to develop differing fruit qualities, scab resistance to scab was noted, and the need to identify which Vf-resistant possessors are durably resistant to scab remains. In 2007, we identified scab on M. floribunda 821 in a breeding block in West Lafayette, IN. Field evaluations were performed in the following years in this breeding block, and has resulted in the identification of four Vf-resistant cultivars as being susceptible to a new race or races of V. inaequalis. These newly susceptible cultivars include ‘Priistine’, ‘Enterprise’, ‘Jona Free’ and ‘Perlette’. Next, progeny from Crandall’s original crosses, but not ‘Prima’. Laboratory evaluation of pathogenicity by leaf disk assay was consistent with field studies, and suggests that some cultivars may be escapes from this new population of V. inaequalis that can infect Vf-resistant cultivars.

Detection of Colletotrichum cereale specimens from modern and historical collections using culture-independent, real-time PCR methods

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Phytopathology 101:S14

Colletotrichum cereale, the causal agent of anthracnose, is commonly found on cereals, forage and prairie grass, and cool-season turfgrasses. Molecular analysis of C. cereale has revealed considerable diversity within the species, with isolates forming two primary lineages (clade A and clade B). Research of C. cereale is limited by the need to establish pure cultures, a time-consuming process that requires several sub-culturing steps and is often hindered by the presence of faster growing organisms. To test whether C. cereale could be detected directly from DNA extracted from infected plant tissue, two real-time PCR probes specific for clades A and B were developed using the single-copy Apn2 (DNA lyase) gene. Over 730 cultured isolates of C. cereale from turfgrass, prairie, and wheat hosts were screened, along with 23 field samples collected from various grass putters on turf, 32 asymptomatic wheat plants, and 106 herbarium specimens on various host substrates. The probes were 100% accurate for C. cereale detection from cultured samples and for the discrimination of known clade A and clade B isolates (avg. cycle threshold [CT]: A = 28.15; B = 26.82). C. cereale was also successfully detected in a clade-specific manner from diseased field samples and 68–103 year-old herbarium specimens (avg. CT = 24.88 and 34.75, respectively). These probes will be useful for culture-independent, high-throughput molecular analysis of C. cereale populations.

Effects of acute low temperature events on establishment of Erysiphe necator and susceptibility of Vitis species

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Phytopathology 101:S14
The obligate biotrophic pathogen *Erysiphe necator* causes grapevine powdery mildew, and coevolved with its hosts: Vitis species native to eastern North America. When the warm-climate species *Vitis vinifera* (the European grapevine) was exposed to acute cold events, there was a significant reduction in conidial germination, sporulation, and colony development. Our objective was to investigate the effects of similar acute cold treatments using the grape species *V. labrusca*, *V. rupestris*, *V. amurensis*, warm climate species, which represent a gradient of cold-tolerance and climatic range within Vitaceae. Leaves from each species were exposed to 2C for 8 h, incubated for an additional 24 h at 24C for 24 h, inoculated with conidia, and finally incubated at 24C for an additional 44–48 h. Control leaves did not undergo cold treatment. Commassie Blue stain was used in microscopic assessments of conidial development as (i) germ tube only, (ii) primary hyphae or (iii) branched hyphae. Results showed that cooler climate Vitis species, *V. labrusca* and *V. rupestris*, did not demonstrate cold-induced resistance to powdery mildew while *V. vinifera* and *V. amurensis*, warm climate species, did demonstrate cold-induced resistance. *V. riparia*, and *V. cinerea* exhibited a higher basal level of resistance that was nonetheless enhanced by the cold shock treatment.

Achieving sustainable potato production through the use of new potato varieties with reduced fungicide requirements

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Phytopathology 101:S15

One of the primary goals of the potato industry is to enhance sustainability by optimizing the efficient use of agronomic inputs. One approach to achieving this goal is to exploit the higher levels of disease resistance in new potato varieties. The Northwest Potato Variety Development Program (NPVDP) has developed new varieties with higher disease resistance than the standard, Russet Burbank. The objective of this study was to determine the extent to which fungicide inputs for newly released NPVDP potato varieties can be reduced. The performances of five NPVDP varieties and Russet Burbank were evaluated in traditional and reduced-fungicide management programs. Plots were fumigated with metam sodium at zero, low, medium and high rates prior to planting to control Verticillium wilt. Fungicide treatments consisted of a seed treatment, in-furrow treatment and one foliar fungicide application, a seed treatment, in-furrow application and four foliar applications, and no fungicides. Foliar disease severity of early blight and white mold over the season was rated as the relative area under the disease progress curve. Levels of Verticillium wilt were estimated in the field by visually rating plant wilt and soil levels of the wilt pathogens *V. dahliae* and *Colletotrichum coccodes* were assessed by qPCR. Yield data were collected at harvest. Overall results showed that all NPVDP varieties performed well under reduced-fungicide management programs and significantly better than Russet Burbank.

Economic analysis of small plot and on-farm trials for soybean in Iowa

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Phytopathology 101:S15

Fungicide use on soybean has greatly increased in Iowa during the last decade and a growing number of producers each year are applying fungicides to the crop. With numerous fungicides available for soybean producers, it is important to be able to quickly compare and evaluate fungicide efficacies. In Iowa, fungicide trials have largely been conducted either as on-farm research studies using strip test comparisons or as small plot studies. On-farm research arguably has more real life application since larger test strips that span an entire field are used to compare one or two products with a nonsprayed control. In small plot research, the efficacy of many fungicide products is more easily compared by using a small area. The goal of this research is to compare yield responses of on-farm research and small plot research through entire field are used to compare one or two products with a nonsprayed control. Data obtained for these analyses were collected between 2006 and 2010 from on-farm research conducted by the Iowa Soybean Association and Iowa State University, and from small plot trials conducted by Iowa State University by multiple researchers. Mean yield responses in on-farm fungicide trials was 2.42 bushels per acre compared with 1.67 bushels per acre in small plot research. Our aim is to improve how fungicides are evaluated in Iowa and also improve soybean management recommendations for growers.

Quantification of *Cylindrocarpon* sp. in roots of almond and peach trees from orchards affected by Prunus replant disease

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Phytopathology 101:S15

Prunus replant disease (PRD) is a poorly understood soilborne complex that suppresses replanted almond and peach orchards in California. Using culture-dependent and culture-independent approaches, we found *Cylindrocarpon* (Cyl) *macrodidymum* among microorganisms associated with PRD. We developed a qPCR assay to further examine the Cyl-PRD association; a pair of forward primer pair that amplified a 374-bp rDNA fragment from *C. macrodidiyum* was coupled with a specific hydrolysis probe. The assay was optimized and validated using genomic DNA from the target and 70 non-target microorganisms and rootstocks. The lower detection limit was 100 fg Cyl DNA per 25 µl of PCR mix. The assay was used with root samples from replicate healthy and PRD-affected almond and peach trees (in fumigated and non-fumigated plots, respectively) in five California orchards. All orchards were replanted in winter and expressed PRD symptoms the following summer. Root samples were collected on 1 to 5 dates per orchard from Apr.-Sept. of the year trees were planted. In orchards 1-3, Cyl levels were significantly higher in PRD-affected than in healthy roots on some dates (7 of 11 sampling dates), but in orchards 4 and 5 (1 date each) Cyl levels were near the lower detection limit and did not differ in relation to PRD incidence. We conclude that Cyl concentration in roots is positively associated with PRD in some orchards; the relationship may be seasonal, requiring systematic temporal sampling for quantification.

A novel endophytic biocontrol agent of oomycete pathogens with the activity of plant growth promotion, resistance induction and nitrogen fixation

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Phytopathology 101:S15

A novel antagonistic bacterial strain against oomycete plant pathogens (*Pythium ultimum* and *Phytophthora capsici*) was isolated from surface sterilized root of a halophyte, *Rosa rugosa*. On the basis of morphological, physiological, biochemical characteristics and phylogenetic analysis, the strain was found to be a new species of genus *Marteella* and proposed as *Marteella endophytica* YC6887 sp. nov. Strain YC6887 could inhibit mycelial growth of *P. ultimum* and *P. capsici* on yeast casein soybean medium. The incidence of Phytophthora blight on pepper treated with the strain was significantly lower than that of non-treated control in pot tests under greenhouse condition. Antibiotic metabolite produced in culture media was extracted with ethyl acetate and purified by column chromatography. In pot bioassay using Arabidopsis and pepper as host plants to determine plant growth promotion and induced systemic resistance, strain YC6887 could promote the growth of both plants by decreasing of cell suspension to the pot and induce systemic resistance of Arabidopsis to infection by the bacterial leaf pathogen *Pseudomonas syringae* pv. tomato. To examine whether the strain is able to colonize plants endophytically, the gfp gene tagged strain YC 6887 was introduced and observed by fluorescence stereomicroscopy. This gfp gene tagged bacteria colonized intracellular spaces in the leaf of tobacco. Additionally this strain formed nodules on the root of soybean and was found to have nod and nif genes.

Molecular detection of banana bacterial soft rot pathogen, *Dickeya sp.* (*Pectobacterium chrysanthemi*)

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(1) Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China, was accountable for significantly economic losses to banana production. Polymerase chain reaction (PCR) techniques offer advantages over traditional methods of detection and diagnosis. At present, primers for detecting *Dickeya* sp. (*P. chrysanthemi*) are not available. In this study, based on differential in 16S-235 rDNA internal transcribed spacer (ITS) sequences of *Dickeya* sp. (*P. chrysanthemi*) and other bacteria, a pair of species-specific primers, Lf/Lr was synthesized. The specificity and sensitivity of the reaction were tested and the PCR protocols were used to detect diseased plant tissues, irrigation water, and diseased soil samples collected in the field. Specificity was tested against more than eight bacterial organisms associated with banana and the LF/LR primers amplified only a single PCR band of approximately 171 bp from *Dickeya* sp. (*P. chrysanthemi*). The detection sensitivity was determined to be 0.44 fg for pure...
silicates belonging to 22 families promote growth of the model plant Arabidopsis thaliana (1). The primary cause of FHB in Idaho is F. culmorum, whereas in other areas of the country, F. graminearum predominates, especially following corn. Every year, the University of Idaho Extension program plants variety trials in several locations throughout the region, including varieties representative of the barley and wheat varieties present in Southern and Eastern Idaho. During the summer of 2010, varieties in the Idaho Falls trial developed FHB symptomatic wheat heads. Observational analysis revealed the presence of FHB in several hard spring wheat varieties during late July and early August of 2010. Isolates were obtained from FHB infected samples and the primary species causing infection were identified. Initial identification determined the primary causal species to be F. culmorum with minor infection by other species, including F. graminearum. Species prevalence will influence recommended crop rotations and potential economic damage to the local grain industry.

**Volatile-mediated plant growth promotion by Fusarium oxysporum**

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Phytopathology 101:S16

Fusarium oxysporum is a cosmopolitan soil-borne fungus that is known to cause wilt diseases in a large number of crop plants. However, not all F. oxysporum isolates are pathogenic, and some isolates have been used as biocontrol agents protecting plants from other pathogens. We have discovered that certain F. oxysporum strains produce volatile compounds that promote growth of the model plant Arabidopsis thaliana. 47 F. oxysporum isolates belonging to 22 formae specialiae were co-cultivated with A. thaliana seedlings in Petri dishes containing a center partition, allowing only for the exchange of gasses between the two sides of the dish. After 14 days, plant fresh weight measurements revealed seven F. oxysporum isolates that strongly promote plant growth through the emission of volatile compounds. In order to determine the mode of action and perception mechanism by which these volatiles affect plant growth, we studied how A. thaliana mutants, defective in several hormonal regulatory pathways controlling plant growth, respond to these volatiles. We are also studying the cellular and developmental changes of A. thaliana plants in response to F. oxysporum volatiles and identifying those compounds through the use of gas chromatography and mass spectrometry. Identification of fungal volatiles underpinning plant growth promotion and the elucidation of their synthesis and mechanisms of action will help us better understand chemical-mediated plant-microbe interactions in the soil.

**Switchgrass rust epidemics (Puccinia emaculata) in agronomic fields in Tennessee**

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Phytopathology 101:S16

Recently, agronomic switchgrass production has increased due to its usage as a crop for production of cellulosic ethanol. In July and August 2007, Puccinia emaculata was reported to cause rust disease on switchgrass in Tennessee. In this study, four agronomic fields of switchgrass in eastern Tennessee were evaluated to determine the epidemiological characteristics of rust on switchgrass. Individual leaves on twenty-five plants were rated for disease severity each of the four fields once per week over a period of fifteen weeks. Disease progress curves were then developed from disease severity data in order to make comparisons between plots as well as between fields. Rust was first detected in late May. Disease severity progressed exponentially, leveling off in late August to early September. Log phase of disease progression occurred between mid-June to mid-August. Final disease ratings were taken immediately before harvest, revealing an average of greater than five percent of leaf surfaces were infected. Leaves began dying in mid-to-late June, but it is not apparent if death was caused by disease or other factors.

**Establishment of a foundational Federal-academic partnership for the enhancement of forensic plant pathology**

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Phytopathology 101:S16

The U.S. National Biosecurity Defense Plan recognizes the need for robust capability in agricultural biosecurity and microbial forensics. A collaborative partnership between the National Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB) and the National Bioforensics Analysis Center (NBFAF) builds strengths in forensic plant pathology. NBFAF’s forensic range is expanded by collaborative research with NIMFFAB to develop and validate real time PCR assays for forensic applications to plant pathogens. As an NBFAF Spoke Laboratory, NIMFFAB provides a link with the plant pathology community through the American Phytopathological Society’s Microbial Forensics Interest Group, in which academic, industry and Federal phytopathologists and security personnel interact and provide input to programs and priorities. As a part of Oklahoma State University, NIMFFAB prepares graduate students through coursework, research and internships to meet future staffing needs of security agencies. Finally, NIMFFAB conducts focused outreach and training to increase awareness and appreciation among the plant pathology and security communities for their respective roles in preventing and responding to potentially criminal plant health emergencies. The NIMFFAB-NBFAF relationship enhances the security of our nation’s plant resources and agricultural enterprise, and demonstrates the potential for multi-disciplinary collaborative efforts between academia and government.

**Gene trees versus species trees for resolving the Phytophthora Clade 1C phylogeny**

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Phytopathology 101:S16

Phytophthora Clade 1C contains foliar blight pathogens, including the causal agent of potato and tomato late blight, P. infestans. Four of the five species within this clade share some sympatrically in the central highlands of Mexico; a fifth species, P. andina, has recently been described from the Andean highlands of Ecuador. These species are found on specific plant family hosts, although P. infestans and P. andina infect diverse sections of the Solanaceae. Due to their rapid and most likely recent diversification, resolving the branching order among the Clade 1C species is challenging. Here we have used sequence data of seven nuclear and six mitochondrial loci, including intron and spacer regions, from 58 isolates to reconstruct a robust species tree. We have also used computational and experimental methods to identify putative incompatibility loci within the heterothallic members of this group. Although phylogenetic signal is low, we have used maximum likelihood methods to test several hypotheses for the temporal diversification of this important group.

**Comparison of nine PCR primer sets designed to detect Pantoea stewartii subsp. stewartii in maize**

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Phytopathology 101:S16

Pantoea stewartii subsp. stewartii, the causal agent of Stewart’s bacterial wilt of maize, is a major quarantine pest in maize seed. Verifying freedom from P. stewartii remains a significant hurdle in exporting corn seed from the U.S. Several PCR primer sets have been developed and suggested as being potentially useful for routine seed health testing. Nine published PCR primer sets were evaluated for their ability to specifically detect P. stewartii and for potential cross reactivity (false positives) with other Pantoea species. Six conventional PCR primer sets and three real-time TaqMan primer sets were compared using Pantoea isolates that included P. stewartii, P. agglomerans, P. ananatis from multiple hosts, and non-P. stewartii isolates from maize. The primer sets targeted regions within the efp gene cluster, hpsG gene, 16S rRNA ITS region, and the pstS-glnM5 region. None of the primer sets was 100% specific for P. stewartii exclusively. Each primer set amplified DNA from additional isolates that included P. ananatis and/or Pantoea (non-P. stewartii) isolates from maize seed. These results suggest that these primer sets may not be suitable for use in routine testing for P. stewartii in maize seed due to the potential for false positive reactions.
**Pestalotiopsis and Colletotrichum species causing latent infection on persimmon fruits in Brazil**

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Phytopathology 101:S17

Persimmons grown in Brazil have suffered an anthracnose disease (Colletotrichum gloeosporioides) causing early defoliation and twig cankers and, recently, a Pestalotiopsis sp. was also identified causing twig and branch cankers. Fruits appear damage at harvest and postharvest storage. In addition, unripe, putatively infected fruits drop before harvest. The objective of this study was to evaluate the incidence of latent infection of both pathogens in unripe persimmon fruits. The level of latent infection was determined using the overnight freezing incubation technique. Samples of 50 unripe fruits of the cultivars ‘Fuyu’ and ‘Kakimel’ were collected in two orchards, first was under an organic and second under a conventional production system. Fruit were collected 120 and 150 days after full bloom. The fruits were surface-sterilized with 92% ethanol, sodium hypochlorite at 400 μg/ml-0.5 mL of Tween 80, and water solution for 5 min. After placing the fruit in a freezer at -15°C for 20 h, the fruits were incubated in a moist chamber at 25 ± 2°C, and evaluated daily for 7 days. The first symptoms of C. gloeosporioides appeared after 3 days while 80% of the Fuyu fruits showed infections after 7 days (both collections). Furthermore, 66 and 87% of the fruit of the first and second collection, respectively, had infections by a Pestalotiopsis sp. However, in Kakimel fruits, 61 and 36% were indicted by C. gloeosporioides and 67 and 19% by a Pestalotiopsis sp., respectively, for the first and second fruit collections.

**Typhula ishikariensis and Typhula incarnata vary in sensitivity to fludioxonil, propiconazole and chlorothalonil**

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Phytopathology 101:S17

High elevation golf courses in Colorado typically treat their putting greens and fairways with preventive fungicides in late October each year to reduce damage caused by gray and speckled snow molds (Typhula incarnata and T. ishikariensis respectively). Over the years, we have observed that applications of chlorothalonil (10–100 μg/g) and fludioxonil (1–5 μg/g), either alone or in combination, fail to provide adequate control of snow molds even though their concentrations in the verdure under snow cover do not diminish significantly during winter. We tested the in vitro sensitivity of 200 T. incarnata and T. ishikariensis isolates to various concentrations of fludioxonil, propiconazole and chlorothalonil. Fludioxonil at 1 μg/g completely inhibited the growth of 95% of the isolates and the rest were inhibited at 10 μg/g. Chlorothalonil at 1–10 μg/g inhibited or significantly reduced the growth of the majority of T. ishikariensis but not T. incarnata isolates. Growth of all but three isolates was inhibited by more than 80% at 10 μg/g propiconazole, but growth of many isolates was unaffected at 1 μg/g. These results indicate intra- and interspecific variability in sensitivity to these fungicides, but do not explain why they are not more effective in the field.

**Onion cultivar resistance to Iris yellow spot virus and onion thrips on physic nut, growth and productivity**

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Phytopathology 101:S17

The tospovirus Iris yellow spot virus (IYSV) and its vector, the onion thrips (Thrips tabaci) are yield-limiting pests of onions in all onion production regions where these pests are prevalent. The polyphagous vector damages stems, flowers and roots, and transmission of the virus occurs in the feeding process and by transmission of the virus in infected bulbils. The feeding habit adversely affects the photosynthetic capacity of plants. In 2009 and 2010, greenhouse experiments were conducted to investigate the effects on onion physiology, growth and productivity of 4 treatments: thrips only (T), IYSV only (V), Thrips+IYSV (TV) and a healthy control (HC) for a field resistant variety, Colorado 6 and a susceptible variety, Talon. Net photosynthesis (Pn), stomatal conductance and intercellular CO2 content showed a decreasing pattern for all treatments throughout the season but the rates were generally higher in Colorado 6 than in Talon. In both varieties, Pn taken at different PAR at 127 DAS showed a similar trend for all treatments but was significantly lower for V. There was no variety difference for both bulb weight (P = 0.9073, F = 0.01) and bulb size (P = 0.0734, F = 3.30). However, there was significant treatment difference for bulb weight (P < 0.0001, F = 49.84) of 57.94, 36.77, 20.12 and 13.99 g bulb, respectively, for HC, V, T and TV. There was also a significant bulb size difference between the treatments (P < 0.001, F = 22.92) with HC having the largest bulb size of 4.50, followed by V, then T and finally by TV with sizes 4.11, 3.48 and 3.07 cm diameter, respectively.

**Activity of citrus canker lesions on leaves, shoots and fruit of grapefruit in a Florida orchard from June 2010 to January 2011**

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Phytopathology 101:S17

Lesions of citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), on citrus fruit preclude export to certain markets. Characterizing the population dynamics of bacteria in canker lesions in commercial orchards can help gauge risk associated with diseased fruit entering fresh markets. The aim of this study was to quantify and compare lesion activity in citrus tissues up to harvest in an east central Florida grapefruit orchard. Each month from June 2010 to January 2011, twenty, fifty and eighty lesions were sampled from shoots, leaves and fruit, respectively. Lesion activity was quantified by dilution plating on nutrient agar and bioassay by injection infiltration into leaves of cv Duncan grapefruit. From June 2010 to January 2011, linear regression analysis indicated a decline in the proportion of active lesions for fruit from 98% to 6% (R² = 0.80), for leaves from 100% to 66% (R² = 0.44) and for stems from 45% to 10% (R² = 0.41). Lesion activity was most erratic for stems. The maximum bacteria flux density (BFD, mm⁻² min⁻¹), a measure of inoculum production was 2.7 ± 105, 2.4 ± 105 and 1.4 ± 104 bacteria mm⁻² min⁻¹ for fruit, leaves, and stems, respectively. Although the greatest reduction in BFD occurred for fruit lesions, considering lesions are active up to the point of harvest there is a role for postharvest disinfection treatments to mitigate Xcc on fresh fruit.

**Evaluation of phosphate to control scab on pecan in the southeastern U.S.A.**

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Phytopathology 101:S17

Pecan scab (caused by Fusarium effusum) infects pecan and severe disease can cause yield loss. The efficicy of phosphite, an elicitor of systemic acquired resistance (SAR) was evaluated in field experiments in 2009 and 2010. Biweekly applications of phosphite (Prophyta at 2.64 L 1000 L⁻¹ ha⁻¹) were compared to an industry standard fungicide, triarylhydrazine (TPH, Super-tin at 0.90 L 1000 L⁻¹ ha⁻¹). Both phosphite and TPH reduced scab provided equally good control of leaf scab, with the exception of one of the TPH treatments in 2010. Phosphite and TPH also gave equally good control of scab early in fruit development (Jul/Aug); however, by the final assessment (Sep/Oct), fruit scab severity on phosphite treated trees was greater than those receiving TPH and in 2010, scab severity was equivalent to the untreated control. There was no difference in fruit volume between phosphate and TPH-treated plots in 2009, and no difference in nut volume between treatments in 2010, although there were treatment differences in kernel weight and fruit weight in 2010. Results indicate that phosphite provides useful control of pecan scab on both foliage and fruit early in the growing season, but does not provide as good prolonged late-season protection compared to an industry standard, TPH.

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Continued deployment of moderate resistance to Cephalosporium stripe in Kansas winter wheat cultivars
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Phytopathology 101:S18

Cephalosporium stripe was a significant problem in Kansas during the 1970s because of the widespread planting of highly-susceptible cultivars. However, beginning with the release of Arkan in 1982, Kansas has had a history of releasing cultivars with moderate levels of resistance. As a result, stripe has declined to trace levels. Because of the virtual disappearance of stripe, little conscious effort is currently placed on developing cultivars with resistance. Due to the lack of emphasis, it is important to periodically test the reaction of cultivars to stripe. Therefore, six winter wheat cultivars were tested in the field to their reaction to stripe. Sturdy was the susceptible check and Plainsman V the moderately-resistant check. Karl 92 was released in 1992, Jagger in 1994, Fuller in 2006, and Everest in 2009. Karl 92, Jagger, and Fuller have been very popular and risen to be the number one cultivar in Kansas. In the field, the susceptible cultivar Sturdy showed 77.6% whiteheads and 51.7% yield loss while moderately-resistant Plainsman V had only 25.4% and 24.1%, respectively. All of the popular Kansas cultivars had disease severity and yield loss values which were equal to or less than Plainsman V. Thus, moderate levels of resistance to stripe appear to have been maintained in cultivars popular in Kansas during the past 20 years including the two most recent releases from Kansas State University (Fuller and Everest).

Multiplex detection of Phytophthora: Padlock probe based Universal detection Multiplex Array (PUMA)
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Phytopathology 101:S18

Phytophthora spp. is responsible for many diseases worldwide, can occur on a wide range of different crops and the number of species is increasing. To detect and identify those several species several molecular methods have been developed for single species only. A uniform method for detection of all Phytophthora species would be very useful for research and regulatory communities. Therefore we developed a diagnostic method to detect a range of Phytophthora species, including P. ramorum. The method includes a generic TaqMan PCR amplification method for all Phytophthora species combined with species specific padlock probe (PLP) detection on a dedicated universal micro-array. Twenty-three padlock probes for 22 Phytophthora species relevant for the Netherlands were developed based on sequence differences in the ITS-1 region. After point mutation specific ligation of a mixture of the 23 PLPs on the generic amplicon, exonuclease treatment to degrade the unreacted probes, amplification of the ligated probes and hybridization on a micro-array, a unique signature on the micro-array can be obtained for each Phytophthora species included in the test. In this paper the specificity and sensitivity of a padlock based diagnostic tool is combined with a cost effective microtiter plate array detection device and has been evaluated using reference Phytophthora cultures as well as mixed infected material collected from field surveys, including air-, root-, water- and plant tissue samples.

Influence of defective RNAs of Tomato black ring virus on symptoms expression
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Phytopathology 101:S18

Tomato black ring virus (TBRV) infects a wide host range, including vegetables, grapevine, soft fruits (raspberry, strawberry), fruit trees and non-cultivated species. TBRV genome consists of two positive-sense, single-strandedRNAs of about 7500 and 4500 nucleotides for RNA1 and RNA2, respectively. A small additional satellite RNA occurs in some isolates. The existence of defective RNA molecules (DI) associated with TBRV has also been shown. D-RNAs frequently from virus genome during prolonged incubation in a host species and affect on symptoms. We collected several isolates of each Phytophthora species and strain. The goal of our work was to confirm whether defective particular act as silencing factors during viral infection. For each isolate originally devoid of defective RNA serial passages in Chenopodium quinoa were performed. After each passage cycle virions purification and RNA extraction was performed. The DNA was isolated from purified viral particles of all isolates after 6, 10, 15 and 20 passages. Analysis of viral RNA revealed the presence of an additional, small RNA segments in the three Polish isolates collected from Robinia pseudacacia and tomato after 15 passages. No additional bands appeared from isolates collected from zucchini and potato. Those isolates in which small genomic RNA segments were generated induced milder symptoms in comparison to TBRV without D-RNAs. This findings can be used in developing new strategies for plant protection.

Fusarium ear rot pathogens and their mycotoxins associated with South African maize
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Phytopathology 101:S18

Maize is the most important agricultural crop produced in Southern Africa, and is consumed daily by millions of Africans as a staple food. In South Africa, the crop is often affected by ear rot pathogens belonging to the genus Fusarium and their mycotoxins. To determine the prevalence of Fusarium species and their toxins, samples were collected from two susceptible maize cultivars at 14 localities in South Africa during 2008 and 2009. Fusarium species was quantified by real-time PCR and their mycotoxins by multi-toxin analysis using HPLC-MS. In 2008, F. graminearum was the predominant species in the eastern Free State, Mpumalanga and KwaZulu-Natal provinces, while F. verticillioides was predominant in the Northwest, the western Free State and the Northern Cape provinces. In 2009, maize ear rot infection was higher and F. graminearum became the predominant species found in the Northwest Province. Fusarium subglutinans was associated with maize ear rot in both years at most of the localities, while F. proliferatum was not detected from any of the localities. Deoxynivalenol and zearalenone correlated well with the amount of F. graminearum found in maize grain, fumonisins with F. verticillioides, and moniliformin and beauvericin with F. subglutinans. Our findings suggest a shift in the occurrence of Fusarium species and their mycotoxins in South African maize, which could be contributed to changing agricultural practices and climatic changes in production areas.

Meta-analysis of Solanum resistance gene analogs—towards a comprehensive catalog of R-gene alleles for research and crop improvement
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Phytopathology 101:S18

Wild Solanum species are rich sources of disease resistance (R) genes for improvement of potato, tomato, and eggplant. Comprehensive surveys of Solanum R-gene alleles will enable research on R-gene evolution and provide informed criteria for the use of wild germplasm. Most R-genes encode a nucleotide binding site (NBS). PCR-based approaches targeting NBS-encoding R-gene sequences have been developed, yielding fragments termed ‘resistance gene analogs’ (RGAs) that originate from R-genes. We generated 91 RGAs from a disease-resistant relative of potato, S. bulbocastanum, complementing smaller existing RGA collections from six Solanum species. By combining 236 RGA and 42 cloned R-gene sequences into a single meta-analysis, we generated SolaR80, a comparative sequence-based framework for assigning gene fragments to R-gene lineages and providing evolutionary relationships (and probable orthology) and DNA cross-hybridization results. The SolaR80 framework provides Solanum researchers with a common terminology for cross-species analyses. Our research shows that most R-gene lineages are present in most Solanum species. One R-gene lineage has undergone expansion and diversification in S. bulbocastanum. We hypothesize that this gene lineage is of significance to the health of the species. Our ongoing efforts include a bioinformatics survey of whole genome sequence of tomato and potato and next generation sequencing of R-gene alleles from a broad array of Solanum species.

FRET probe genotyping of Xylella fastidiosa strains
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Phytopathology 101:S18

Epidemiological studies of Pierce’s disease (PD) can be confounded by a lack of genetic information on the bacterial causative agent, Xylella fastidiosa (Xf). PD in grape is caused by genetically distinct strains of Xylella fastidiosa subsp. fastidiosa (Xf), but is not caused by numerous other strains or subspecies of Xf that typically colonize plants other than grape. Detection assays such as ELISA and qPCR are effective at detecting and quantifying Xf presence or absence, but offer no information on Xf subspecies or strain identity. Surveying insects or host plants for Xf by current ELISA or qPCR methods provides only presence/absence and quantity information for any and
all *Xf* subspecies, potentially leading to false assessments of disease threat. This study provides a series of adjacent-binding fluorescence resonance energy transfer (FRET) DNA melt analysis probes that are capable of efficiently discriminating *Xf* subspecies and strain relationships in rapid real-time PCR reactions. These dual hybridization probes are used on *Xf* positive insect and grape DNA extractions to provide *Xf* genotype information, and constitute a Multicolor Melt Typing (MLMT) assay for *Xf*.

**Disease severity and microsclerotium properties of the sorghum sooty stripe pathogen, Ramulispora sorghi**

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Phytopathology 101:S19

Ramulispora sorghi causes sooty stripe of sorghum. Disease severity in irrigated and dryland plots was measured for 25 susceptible genotypes during the 2007 and 2008 growing seasons using a rating scale based upon percent leaf infected. Disease severity ratings were approximately 1.4 points higher (*P* < 0.0001) on the rating scale in the irrigated plots than dryland plots for 2007 and 2008. Sooty stripe lesions were collected from each sorghum genotype in irrigated plots and assessed for mean microsclerotium production within lesions, microsclerotium size, and sporogenic germination, with significant differences apparent between for microsclerotium size (*P* = 0.01) and sporogenic germination (*P* = 0.01). There was no relationship between disease severity and microsclerotium production within leaf lesions, microsclerotium size, or sporogenic germination; however, there was a positive and significant correlation between microsclerotia production within a lesion and microsclerotium size (*P* = 0.019, *P* < 0.0001). Although microsclerotia from sorghum lesions varied in structural characteristics and their ability to produce spore masses, these qualities were dependent upon the sorghum genotype from which the microsclerotia were derived, since the *R. sorghi* population was genetically uniform as determined by ITS sequences and RAPD PCR.

**Comparative genomics of Salmonella enterica serovar Weltevreden plant and animal isolates**

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Phytopathology 101:S19

Salmonella enterica serovars are a prevalent cause of human gastroenteritis linked to fresh plant produce. Salmonella Weltevreden has been a predominant food-safety serovar in South-East Asia associated with animal products. Recently, it has emerged as a global problem also associated with vegetables. We sequenced the complete genome of a *S. Weltevreden* isolate from a Scandinavian outbreak traced to alfalfa sprouts. Illumina and 454 sequencing delivered 18.7Gb/46.4x coverage, with read assembling into 91 contigs. The 4.9 Mb genome has a 52.1% G+C content, 4858 coding sequences (CDS), and a novel plasmid pSW82 (81.9 bp, 93 CDS). Comparative analysis of the plant isolate against a resequenced genome of a scallop isolate revealed intriguing differences despite high overall homology. Analysis of clustered interspaced short palindromic repeat regions (CRISPRs) that record extrachromosomal infections indicate recent divergent evolution of plant and animal isolates. Comparative analysis with other *S. enterica* serovars showed a high synteny in gene content, but revealed notable differences. S. Weltevreden is one of the few serovars to have a type VI secretion system (T6SS) with two functional gene clusters. Differential presence/absence of several phosphotransferase systems (PTS) was revealed, which may enhance survival of S. Weltevreden on a broader range of hosts. Differential transcriptomics analysis in planta is underway and will be discussed.

**Aggressiveness of Rhizoctonia solani AG 2-2 ISGs IV and IIIB on sugar beet and rotation crops**

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Phytopathology 101:S19

Rhizoctonia crown and root rot (RCRR) of sugar beet is caused by *Rhizoctonia solani* AG 2-2 intraspecific groups (ISGs) IV and IIIB. Isolates from field plots with RCRR (24 ISG) were tested for aggressiveness on seedlings and adult plants of sugar beet, pinto bean, soybean, corn, and hard red spring wheat. A commercial greenhouse soil was infested with each ISG isolate before sowing. Two weeks after planting (WAP), seedlings were evaluated with a root rot index (RRI) for sugar beet (0–100 scale); beans and corn (1–5 scale); and wheat (0–3 scale). Adult sugar beet roots were inoculated 8 WAP; bean crops at/near flowering; corn at V5/V6; and wheat at 6 WAP. Two weeks after inoculation, adult crops were rated for disease with the same RRI used for seedlings (except for sugar beet, 0–7 scale). There was significant variability among isolates within ISGs and ranges overlapped. On seedlings, IIIB was more aggressive than IV and average RRIs on sugar beet were 78 and 51, on pinto bean were 4.4 and 2.7, on soybean were 4.1 and 3.2, on corn were 3.1 and 2.1, and on wheat were 1.0 and 0.7, respectively. Aggressiveness was similar for ISGs on adult sugar beet (5.0 for IV, 4.9 for IIIB), pinto bean (2.9 for IV, 3.1 for IIIB) and soybean (3.5 for both ISGs). On corn, RRIs averaged 1.7 for IV and 2.3 for IIIB and on wheat were 0.1 for IV and 0.3 for IIIB. Thus, RCRR of sugar beet is less likely to occur with hard red spring wheat in the rotation compared to beans and corn.

**Pantoea agglomerans** fire blight biocontrol strain- and species-specific real-time PCR tools to monitor environmental impact and behavior in orchards

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Phytopathology 101:S19

**Pantoea agglomerans** E325 (Northwest Agricultural Products, Bloomtime) is a promising biocontrol alternative for fire blight management. To facilitate European registration, we developed quantitative real-time PCR (qPCR) tools for environmental impact assessment. Strain-specific ID based on unique sequences of a 3-kb genomic island discriminated E325 from other *Pantoea* products (Blight-Ban C9-1, BlossomBless P110c). Impact assessment on indigenous *Pantoea* was enabled by species-specific ID (conserved pagRI autoinducer genes) and mechanism-of-action genes (pantocin A; novel E325 antibacterial metabolite). Monitoring in apple orchard trials (2009–2010; 3 locations) validated sensitivity/specificity of qPCR assays (LOD 3 cells/reaction 2.5 CFU/flower). E325 established on 80–100% of flowers, with secondary colonization restricted to treated plots and no escape outside orchards. Population dynamics followed expected models for seasonal low temperatures (stable log 4-5 CFU/flower after 5-6 d). There was no significant impact on (or risk to) native *Pantoea* flower communities. E325 did not persist on foliage or soil after 120 d. No residues were detected on harvested fruit indicating consumer exposure issues. Biocontrol features were rare in native *Pantoea* (eg, pantocin A genes <7.4% frequency). Monitoring results indicate no adverse impact/risk of E325 applied to control fire blight.

**Erwinia amylovora** early detection in orchards using lateral-flow immunostrips Ea AgriStrip and quantitative PCR for flower monitoring

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Phytopathology 101:S19

Fire blight forecasting models predicting infection risk are routinely used to determine timing of application of *Erwinia amylovora* control products. *Ea* AgriStrip is simple to use and suitable for on-site monitoring giving results within 15 min. Depending upon flower sampling scheme, qPCR may be overly sensitive, leading to excessive application of control products, while the sensitivity of *Ea* AgriStrip may better reflect infectious populations warranting intervention.

**Early emergence applications of prothioconazole for management of Cylindrocladium black rot of peanut**

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Phytopathology 101:S19

Cylindrocladium black rot (CBR) of peanut is a disease of roots and pods caused by *C. parasiticum*. Early emergence (EE) applications (2-3 weeks after planting) in an 8 cm band in a high volume (373 L/ha) spray were evaluated in 2006. The EE application followed by four sprays of Provost (0.58 L/ha, prothioconazole + tebuconazole) reduced CBR incidence 18% and increased yield 1042 kg/ha versus the Provost alone. In 2007, both IF and EE applications followed by Provost significantly increased yield in two tests by an average of 672 and 837 kg/ha, respectively, and the final CBR incidence was reduced 46 and 42%, respectively. In 2008, CBR levels were lower, and...
Localization of *Banana bunch top virus* within the aphid vector, *Pentalonia nigronervosa* as revealed by Immunofluorescence, TEM, and PCR assays

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Phytopathology 101:S20

We have used Immunofluorescence, Transmission Electron Microscopy (TEM), and PCR assays to specifically localize *Banana bunch top virus* (BBTV; family Nanoviridae, genus Babavirus) within its aphid vector, *Pentalonia nigronervosa* (Hemiptera, Aphididae). Aphids were exposed to BBTV infected plants for an acquisition access period of 7–10 days; thereafter, guts and salivary glands were dissected and processed for the localization of the virus. BBTV was localized in Immunofluorescence using either monoclonal or polyclonal antibodies into the anterior midgut. TEM observations revealed the presence of vesicles of 0.5–1 μm as diameter containing paracrystalline structures in the cytoplasm of cells composing the anterior midgut. These structures were absent in aphids raised from healthy banana plants. BBTV was localized in Immunofluorescence within the principal salivary glands with no evidence for labelling into the accessory salivary glands. The virus genomic DNA was detected by using a diagnostic PCR from the guts, salivary glands, and hemolymph. A likely path of translocation of BBTV may therefore include the penetration of ingested viral particles through the anterior midgut, hemolymph infection, and the invasion of the accessory salivary glands. An alternative virus translocation path, which may involve the direct penetration of the salivary glands from the anterior midgut, is suggested because cells forming the principal salivary glands lay in direct contact to the stomach wall.

Mixed modes of reproduction and spatial aggregation of genotypes of the grape powdery mildew fungus, *Erysiphe necator*, within vineyards

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Phytopathology 101:S20

Random mating leads to high genotypic diversity, 1:1 mating-type ratios, and random associations of alleles at different loci, i.e., linkage equilibrium. To test for random mating in populations of the grape powdery mildew fungus, *Erysiphe necator*, we sampled isolates from two vineyards of *Vitis vinifera* in October 2009 (Watkins Glen, NY: N = 62; Winchester, VA: N = 78). The location of each isolate within a vineyard was recorded. We also sampled isolates from the VA vineyard in June 2010 (N = 69). Isolates were genotyped for mating-type and 11 SSR markers. Genotypic diversity was high in all populations. The October 2009 populations contained a large proportion of clones (NY: 35%; VA: 56%) dominated by few genotypes; however, only two clones of the same genotype were detected in the June 2010 population. After clone correction, mating-type ratios in the three populations did not deviate from 1:1. Yet, significant linkage disequilibrium was detected in all three populations even after clone correction. Mantel tests resulted in positive correlations between genetic and geographic distances within vineyards demonstrating that similar genotypes were spatially aggregated and that inoculum disperses short distances. These results suggest that clonal selection and spatial aggregation contribute to linkage disequilibrium even though the population undergoes an annual sexual cycle.

Pest interceptions on live plants at U.S. ports of entry: A system overwhelmed

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Phytopathology 101:S20

More than half of the non-native forest pathogen species in the U.S. today arrived via the plants for planting pathway, often with disastrous ecological consequences. Currently, U.S. regulations rely on 1) phytosanitary certificates issued by the exporting country’s national plant protection organization, and 2) inspections at certain ports of entry with plant inspection stations. Plant imports increased 500% since 1967, with 2.5 billion plants imported in 2010. About 56 inspectors are employed nationwide to inspect live plants. We examined two data sources, both maintained by the USDA Animal and Plant Health Inspection Service; to estimate the approach rate of plant pests (insects and pathogens) in shipments of live plants for propagation entering the U.S., and determined the inspection efficiency of port inspections.

Under normal port operations, only 2.3% of plant shipments were identified as containing actionable pests. In contrast, the Agricultural Quarantine Inpection Monitoring System dataset, a random subset of much more intensively inspected shipments, identified 9.9% of incoming shipments as infested with actionable pests. This discrepancy suggests that at least 76.5% of incoming infested plant shipments pass the ports undetected. The International Plant Protection Convention is developing a standard to harmonize regulations of plants for planting. These data suggest it would be wise to limit reliance on inspections at ports of entry.

Population structure of *Ophiognomonia clavigignetti-juglandacearum* reveals multiple introductions of the butternut canker fungus into North America

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Phytopathology 101:S20

Butternut canker caused by the fungal pathogen *Ophiognomonia clavigignetti-juglandacearum* (Oc-j) remains the primary cause for range-wide mortality of butternut. The disease was first reported in Wisconsin in 1967, however the fungus may have been present for several years prior. Therefore, our objectives were to evaluate the genetic diversity of isolates of Oc-j recovered from butternut, black walnut, and heartnut in North America, and to correlate these results with selected assessments of host range and virulence to identify possible associations among these variables. A total of 48 isolates of Oc-j from across North America including isolates from black walnut and heartnut were analyzed. Clustering analyses based on 16 SNP markers revealed that Oc-j populations are made of four differentiated genetic clusters. This result suggests multiple introductions of the pathogen through successful or simultaneous introductions of isolates having differentiated genetic backgrounds. Isolates recovered from heartnut and black walnut caused larger lesions on all three Juglans species compared to isolates originally recovered from butternut. Eight of the nine isolates, which caused the largest lesions on butternut belonged to a single genetic cluster. The pathogenicity data in combination with geographic and population structure data indicate this fungus was introduced into North America on multiple occasions, and genetic clusters differ in their level of virulence.

Effect of post-inoculation relative humidity on peanut infection by *Sclerotinia minor*

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Phytopathology 101:S20

Stems of six-week-old plants of the cv Okrun (susceptible to *Sclerotinia blight*) were inoculated with *S. minor*. Two post-inoculation humidity regimes of 100% RH were used. In the first RH regime, one inoculation chamber was kept open for the duration of experiment (DOE), and five were closed for durations of 1, 2, 3, 4 or 7 days post-inoculation (PI). In the second RH regime, one chamber was kept open for the DOE, and five were closed for durations of 12, 24, 36, 48 and 60 hour PI. No infection occurred in chambers opened for the DOE or closed for 12 hr. Closure for 24 hr resulted in 50–75% infection, and closure for 48 hr or more resulted in 88–100% infection. Lesions on infected stems were measured up to 7 days after inoculation to calculate area under lesion expansion curve (AULEC). Closure for 24 hr produced AULEC of 8.2–9.7 cm², whereas significantly (P = 0.05) higher AULEC of 18.0–16.0 cm² were obtained with closure of ≥48 hr. These findings indicate the importance of providing 100% RH for at least 48 hr post-inoculation to effectively quantify lesion expansion.

Characterization of two newly described Curtovirus isolated from spinach in south-central Arizona

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Phytopathology 101:S20

A commercial spinach field in south-central Arizona developed geminivirus-like disease symptoms. Total DNA extracts was used as template for rolling circle amplification (RCA) of the viral genomes. RCA products were digested and cloned using PstI to obtain full length curtoviral genomes. A first curtovirus consisted on 3065-bp in length and it was tentatively named Spinach severe curly top virus-[AZ] (SSCTV-[AZ]). An ClustalV alignment with sequences of all curtovirus species available in GenBank indicated that this spinach isolate shared the highest nt sequence identity at 59% with Horseradish curly top virus (HrCTV). The genome consists of six open reading frames and lacks the AC3 gene, an arrangement most similar to HrCTV. A second curtovirus consisted on 2860-bp in length and it was provisionally named Spinach curly top Arizona virus
A vector for virus induced gene silencing (VIGS) based on the Cotton leaf curl virus (CLCrV) DNA-A, was used to demonstrate the use of VIGS for transient gene silencing of different selected cotton genes: 1) fatty acid desaturase (FAD2-4), 2) a steroid 5x-reductase (GhDhET2), and 3) chitinase like genes (GhCHT1 and GhCHT2). Total RNA was isolated from cotton (leaves, roots, cotton ball) and used as template to amplify (by RT-PCR) different size length fragments using specific primers for each gene of interest. These fragments were cloned into the VIGS CLCrV vector. VIGS CLCrV vectors carrying FAD2-4, DET2, CTTL and DET2 were used to inoculate cotton seedlings (Gossypium hirsutum cv Deltapine 5415) with 1.1-µm diameter tungsten microprojectiles (BioRad) coated with a mixture of 1 µg each of the following plasmids. Inoculated plants were kept in the growth room for 2 days. Mean lesion length was measured for controls. Results showed that cotton plants inoculated with the CLCrV – FAD2-4, CTTL and DET2 VIGS vectors and the CLCrV DNA B dimer developed mild CLCrV symptoms at four weeks post inoculation. All vectors were detected successfully replicating in cotton, analysis of FAD2-4, CTTL and DET gene expression confirmed the silencing of these genes in cotton.

Screening Taro (Colocasia esculenta) for resistance to Taro Leaf Blight (TLB) using a detached-leaf disc bioassay and marker-assisted selection

Taro (Colocasia esculenta) is a tropical root crop cultivated primarily for its starchy crown. A major disease that threatens the sustainability of taro is Taro Leaf Blight (TLB) caused by the oomycete pathogen Phytophthora colocasiae. While Hawaiian varieties of taro that are susceptible to P. colocasiae, tolerance to TLB has been found within taro germplasm from Palau, Thailand, and Guam. Hybrid lines can be tested for tolerance to TLB using a detached-leaf disc bioassay. Four 36 mm discs are cut from the first fully developed leaf of each hybrid. Discs are inoculated with approximately 50 zoospores of a local isolate of P. colocasiae and incubated at 27°C in a humid chamber. Lesion length is measured on day three and four for each hybrid. Preliminary analysis showed approximately 25 hybrids that were highly tolerant to TLB. The majority of the tolerant hybrids were a cross between Dirrategadik/Moi and (Red Moi/PH15)/Sawahn Kurasae. When the hybrid screening is complete, 150 of the most tolerant and 150 of the most susceptible hybrids will be transferred into the field for further analysis. Additionally, genetic markers are needed for taro germplasm characterization and to accelerate the resistance screening for early selection of desirable hybrids. Both microsatellites and SNPs are being evaluated for use in marker-assisted selection.

Proteins associated with aflatoxin-resistance are identified and characterized towards candidacy for breeding markers

Aflatoxins, the toxic and carcinogenic secondary metabolites of Aspergillus flavus and A. parasiticus are produced during infection of maize, causing serious preharvest and postharvest problems. Host resistance has become a viable approach to controlling aflatoxin contamination since the discovery of virion-like and sputum-like particles in the SCLAV genome. The virion-like particles are constructed and agroinoculated alone and in mixture to Nicotiana benthamiana, seedlings to test infectivity.
The Oomycota Pythium oligandrum is a biocontrol agent which is known for its ability to induce plant resistance and to control pathogens in the rhizosphere. For instance, this antagonistic oomycete produces 3 elicitin-like proteins (oligandin POD1, POD2) which induce resistance when they are applied on plants. In the present experiment, P. oligandrum strains were isolated from various areas of the Bordeaux vineyard. Subsequently, the strains were characterized and tested for vine protection against an Phaeomoniella chlamydospora. Roots were sampled from vine plants grown in various soils (silt-clay, sandy-clay, stony) from the Bordeaux vineyard region. Whatever the conditions, P. oligandrum strains were isolated from nearly all the samples. It seems therefore ecologically adapted to vineyard soils. Forty strains were collected, purified and identified by sequencing of the rDNA ITS. P. oligandrum elicitor detections were carried out by amplification of oligandin and POD1 genes. These genes were detected and sequenced in most of the strains, Inter Simple Sequence Repeat were used to assess genetic diversity too. A greenhouse experiment pointed out that Cabernet Sauvignon infections by P. chlamydospora were protected up to 50% when the rhizosphere of vines was colonized with P. oligandrum. Taken together these results provide useful information for screening and developing of P. oligandrum biocontrol strategy.

The role of mycotoxins produced by Fusarium verticillioides and Fusarium graminearum in maize seeding infection
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Phytopathology 101:S22

Fusarium verticillioides and Fusarium graminearum are fungi that produce mycotoxins (fumonisins and deoxynivalenol, respectively) and affect plant health by causing disease. While it is well known that these mycotoxins reduce the value of contaminated crops as food or feed, their impacts on seedling health have not been fully described. In order to characterize these effects on seedling infection and disease, wild-type and mycotoxin-nonproducing mutant strains of each fungus were grown on washed and sterilized rice hulls and mixed with sterile vermiculite. Maize seeds were planted into the infested vermiculite and harvested after 8, 14 and 21 days. Root and shoot weights and shoot length were measured and roots were scanned for image analysis of root structure and symptoms. Crown tissue was excised, surface sterilized and used for fungal isolation and quantitative PCR to determine fungal DNA present in the tissue. For F. verticillioides there were significant differences between the fumonisin and non-fumonisin producers for all the measured variables by 14 days after planting. Plants exposed to the fumonisin-knockout strain had longer shoot lengths (p < 0.0001) and higher root (p = 0.0026) and shoot weights (p = 0.0004) and less fungal DNA (p < 0.0001) present in the tissue than plants exposed to the wild-type. For F. graminearum, seedling disease symptoms and infection were similar for the wild-type and trichothecene knockout strains.

First report of the yeast Eremothecium coryli associated with brown marmorated stink bug feeding injury on tomato and apple
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Phytopathology 101:S22

An outbreak of brown marmorated stink bug (BMSB) (Halyomorpha halys), an exotic species first found in the U.S. in 2001, occurred in several mid-Atlantic states in 2010, causing damage to tree fruit and vegetables. Feeding damage to apple fruit consisted of slightly sunken areas approximately 0.3–0.6 cm in diameter. In tomatoes, the feeding sites appeared as irregular yellowish spots on ripe fruit. Damaged apple and tomato fruit were surface disinfested with 95% ethanol, the outer epidermis removed aseptically, and the discolored internal tissue plated on agar. A yeast was recovered from all tissue pieces after 4 days. The isolates were identified by morphology and 28S rDNA analysis as Eremothecium coryli, a plant pathogenic yeast associated with native stink bug species. To confirm transmission of E. coryli by BMSB, fifty adults were captured and enclosed in a screen cage in the greenhouse. Unblistered apples and tomatoes were placed in the cage and after 7 days examined for symptoms. Feeding injury similar to that seen in the field occurred on all fruit and E. coryli was recovered from all lesions tested. To our knowledge, this is the first report of this yeast associated with H. halys. Studies are underway to determine the role of this yeast in fruit damage and how to best manage its transmission.

Managing daylily rust with fungicide dips, drenches and foliar spray applications
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Phytopathology 101:S22

Daylily rust, caused by Puccinia hemerocallioidis, was introduced into the U.S. in 2000 and continues to be problematic to growers in southern production areas. Experiments were conducted to assess the efficacy of various fungicides applied as root dips, drenches, or foliar sprays to manage daylily rust. Root dip treatments included six fungicides and sodium hypochlorite. Single drench applications of four fungicides (azoxystrobin, fluoroacetin, tebuconazole) at three rates (0.06, 0.13, and 0.25 g a.i./plant) were evaluated in greenhouse trials and two fungicides (azoxy- strobir and tebuconazole) at one rate (0.15 g a.i./plant) were evaluated in the field. Eight foliar treatments were applied at 14-day intervals in the field. Dip treatments with azoxystrobin significantly reduced disease development. All drench treatments, except myclobutanil at 0.06 g a.i./plant, significantly reduced rust development in potted daylily when plants were challenged inoculated 3 weeks post-treatment. Drench or foliar applications of azoxystrobin provided the greatest suppression of rust development on daylily in the field. One drench application of azoxystrobin protected plants from rust for over four months. Fungicides dips and drenches could minimize the threat of accidentally introducing daylily rust on propagative materials to new locations and reduce disease development in established field plantings.

Managing resistance of Cercospora beticola Sacc for integrated disease management in sugar beet
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Phytopathology 101:S22

In 2010, a field trial with artificial inoculations was conducted in order to evaluate the efficacy of flutriafol (DMIs) and carbedazim (MBCs) based fungicides for the control of Cercospora leaf spot. Individual plots were inoculated with isolates: a) sensitive to flutriafol and carbendazim, b) resistant to flutriafol and sensitive to carbedazim, c) resistant to flutriafol and carbedazim and d) sensitive to flutriafol and resistant to carbedazim. A total of four fungicide applications were made and three disease evaluations were performed during the growing season. Additionally, at harvest, yield and sugar content were calculated. The highest disease intensity was detected in inoculated plots without fungicide applications and in treatments inoculated with resistant isolates plus fungicide applications. Root yield and sugar content were significantly higher in plots inoculated with sensitive isolates and subsequently treated with fungicides. Detection of fungicide resistance and its management is an important tool in disease control.

Blue-green and chlorophyll fluorescence-based differentiation between simultaneously occurring N-deficiency and pathogen infection in winter wheat
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Phytopathology 101:S22

In recent years several sensor-based approaches have been established to early detect non-destructively single stress factors, but the challenge to discriminate simultaneously occurring stressors still remains. Earlier studies on wheat plants threatened by nitrogen deficiency and leaf rust suggested that the chlorophyll fluorescence might be suited to distinguish between both types of stressors. Nevertheless, there is lack of information on the pre-symptomatic detection of synchronized occurrence of slight N-deficiency and the early stages of pathogen development. The usefulness of the blue, green, and yellow fluorescence signals in this context has not yet been exploited. Based on this, we hypothesized that a differentiation between physiological reaction of wheat plants due to N-deficiency and leaf rust (Puccinia graminis f. sp. tritici) as well as N-deficiency and powdery mildew (Blumeria graminis f. sp. tritici) might be accomplished by means of UV laser-induced fluorescence spectral measurements. Results reveal that both fluorescence amplitude ratios R/FR and B/G enable a reliable and robust discrimination between the experimental groups. Furthermore, the discrimination was done as early as one and two days after inoculation for powdery mildew and leaf rust infection, respectively. Moreover, several additional amplitude ratios and half-bandwidth ratios were suited to early detect the pathogen infection, irrespective of the nitrogen status of the plants.

The use of field bioassay to facilitate the deregulation of fields formerly infested with Globodera rostochiensis in New York
Phytopathology 101:S22
Globodera rostochiensis (Golden Nematode or GN) is a potato cyst nematode, causing much crop damage worldwide and is a quarantine pest in the U.S. due to its exotic nature and its potential for crop damage and trade impacts. First detected on Long Island in 1941, GN has been found on fields in portions of 8 New York Counties with a total of 1.277 million acres under regulation and 6000 acres known to beinfested with GN. The PPQ/NYDAMPGN Program has been in place since 1948. Until recently there have been few mechanisms to releaseinfested potato fields from regulation without removing them completely from host production. A bilateral agreement between the USDA APHIS PPQ and the Canadian Food Inspection Agency was recently adopted that allows for the release of fields formerlyinfested with potato cyst nematodes. A combination of repeated field surveys, cyst population suppression, time and finally challenging the remaining cysts in the field to the repeated presence of host plant material (a bioassay) are utilized to determine if any of the remaining cysts in the field are viable. The first fields for bioassay have been out of host crop production for nearly 30 years and have not had viable GN cysts detected for a minimum of 10 years. The bioassay includes planting susceptible host crops in fieldfoci for 3 years followed by soil sampling to a depth of 25cm obtaining 9 2000ce samples per acre. Fields may be partially released based on negative bioassay results.

The role of an oxidative stress sensor in the oxidative stress response, virulence and host colonization of Pantoaea stewartii subsp. stewartii
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Phytopathology 101:S23

Pantoaea stewartii subsp. stewartii, the etiological agent of Stewart's wilt, is a serious pathogen of sweet corn. An important aspect of bacterial plant colonization is the ability to withstand exposure to reactive oxygen species (ROS) arising from the host defense response or normal plant developmental processes. The transcriptional regulator, OxyR, modulates the oxidative stress response in many bacteria through production of ROS detoxifying enzymes and other important bacterial survival mechanisms such as biofilm formation. A P. stewartii ΔoxyR mutant showed increased sensitivity to ROS and changes in OxyR-dependent catalase expression. Moreover, the ΔoxyR mutant displays a marked decrease in the production of stewartan exopolysaccharide, a key component of the mature biofilm matrix, which is essential to the infection process. The ΔoxyR mutant was less virulent in sweet corn, but was capable of colonizing plants at levels twice as high as wild type. Following treatment with ROS, ΔoxyR showed a striking increase in expression of soxS, part of a second oxidative stress response pathway (SoxRS). We hypothesize that there is partial overlap of the OxyR and SoxRS regulons and that P. stewartii compensates for the lack of OxyR by upregulating the SoxRS pathway, causing the increase in host colonization. Further characterization of the OxyR regulon of P. stewartii will provide insight into the vulnerabilities of xylem dwelling bacteria during plant colonization.

Influence of Pythium aphanidermatum, P. irregulare, and P. cryptóirregulare on the bacterial community in recycled irrigated water
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Phytopathology 101:S23

Pythium species are among the most damaging pathogens in horticulture causing damping-off, root and stem rots in ornamental plants. They have been recovered from recycled irrigated water in commercial greenhouses in PA. An understanding of the interaction among these oomycetes and the microbial community present in the greenhouse system is vital for establishing a long term management strategy. Little is known about the impact that Pythium spp. have on the bacteria communities in recycled water reservoirs. Responses of bacteria communities changes may be associated to the presence of Pythium species in the water. A study showed that Pythium ultimum favors specific genera such as Actinobacteria, Proteobacteria, Chytridiomycota and Sordariomycetes in compost whereas compost without P. ultimum was dominated by Homobasidiomycota. Culture-independent techniques such as automated ribosomal intergenic spacer analysis (ARISA), have expanded our understanding of the diversity of bacteria populations in various ecosystems including soil and water. This study is examining the impact the presence of P. aphanidermatum, P. irregulare, or P. cryptóirregulare has on bacteria diversity and community composition in water in order to develop a better understanding of the greenhouse ecosystem. Profiles obtained using ARISA will help us assess the community-specific profile for three Pythium species. Preliminary results suggest that Pythium presence might play a role in structuring bacterial community composition.

Induction of grape tissue necrosis and tobacco leaf HR by Agrobacterium vitis requires a polyketide synthase and a nonribosomal peptide synthase
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Phytopathology 101:S23

Mutation of an Spf-type of phosphopantetheinyl tansf erase (PPTase) gene (Avi5813) in chromosome of Agrobacterium vitis strain F2/F5 leads to modifications of multiple phenotypes including the hypersensitive response (HR) in tobacco and necrosis in grape. Complementation with the full length of PPTase gene fully restored the HR and necrosis responses. Screening of the A. vitis strain F25 genome revealed a single copy of Spf-type PPTase gene that must be involved in polyketide and non-ribosomal peptide biosynthesis. Mutagenesis of the genes encoding proteins that require post-translational modification by PPTase resulted in identification of a type-I polyketide synthase (PKS) gene (Avi4330) and a non-ribosomal peptide synthase (NRPS) gene (Avi3342) that are both required for induction of HR and necrosis. Knockout of either Avi4330 or Avi3342 caused a necrosis and HR minus phenotype regardless of cell concentration or culture age. This result suggests that an effector molecule required for induction of HR and grape necrosis is likely a hybrid peptide-polyketide product.

Preceence and levels of aflatoxins in common bean (Phaseolus vulgaris L.) samples from Uganda
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Phytopathology 101:S23

Aflatoxins are produced by Aspergillus flavus which grows on a variety of grains and in humans are associated with aflatoxicosis. Occurrence and extent of contamination is influenced by environmental factors and vary with growing location, agro-economic practices, and conditions during pre-harvest and/or post-harvest processing. This study was aimed to assay the presence and levels of aflatoxins on common bean grain samples from different growing and storage conditions in Uganda. Grain samples were collected from markets and farmers’ stores in three districts of Uganda. More than 51% of the collected seeds had been stored between 2 to 5 months. Each of the samples was subjected to standard seed health tests and also screened using a one-step lateral flow immunochromatographic assay with a cut-off of 4 ppb using AgraStrip® Aflatoxin Test kit. Positive samples with aflatoxin greater or equal to 4ppb were subjected to an ELISA-based quantification procedure. Seed health tests revealed 15 species of fungal contaminants on the seed samples with Aspergillus flavus occurring on more than 96% of the samples. Qualitative aflatoxin tests detected over 10% positive cases (1.9 to 24.8 ppb). Most of the positive cases and highest levels of contamination were from the warm areas while samples from the cooler areas had levels below the detection level. Further extensive assessment is planned to establish the extent and significance of aflatoxins on the safety of bean grain used for human consumption.

Arbuscular mycorrhizal fungal diversity associated with coexisting cheatgrass and big sagebrush communities
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Phytopathology 101:S23

Arbuscular mycorrhizal fungi (AMF) are important plant symbionts, and have been implicated in successful plant invasions through multiple mechanisms. Very little is known about the role of AMF on cheatgrass (Bromus tectorum) invasion and persistence. Cheatgrass has been shown to reduce the AMF diversity of neighboring vegetation (Hawkes et al. 2006), and soils from cheatgrass dominated areas have lower mycorrhizal inoculum than unveaded soils (Al-Qawari 2003). However, the identities of AMF species associated with cheatgrass, as well as the diversity of cheatgrass-AMF associations are largely unknown. On the other hand, big sagebrush (Artemisia tridentata) has been shown to have high diversity of associated AMF (Allen et al. 1995). An important question in understanding replacement of big sagebrush dominance by cheatgrass is how this shift alters the diversity of AMF across the sagebrush-steppe. The research presented here compares diversity of AMF associating with coexisting cheatgrass and big sagebrush. A MF species were identified from three distinct locations in Colorado, Utah and Wyoming using trap cultures containing field-collected soil and root material grown for one year in a greenhouse. Diversity was also measured using DNA extracted from the same soil and root samples. DNA was amplified using AMF-specific primers, cloned and sequenced. The understanding of key plant-microbe interactions like AMF will improve effective management of invasive plants.
Preliminary results of the distribution and genetic diversity of Potato virus Y (PVY) in the main Turkish pepper growing areas

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Phytopathology 101:S24

Potato virus Y (PVY) is the type member of the genus Potyvirus (family Potyviridae), the largest group of RNA plant viruses. PVY isolates were collected according to hierarchical (nested) sampling design. Collections were made in 100 fields in seven localities in Hatay, thirteen localities in Kahramanmaraş (Eastern Mediterranean) and seven localities in Gaziantep (Southwest Anatolia). DAS-ELISA revealed the infection of 167 samples with PVY from a total of 1098 pepper (Capsicum annuum) plants showing symptoms. Genetic diversity among these 167 samples was examined by RT-PCR-RFLP analysis of coat protein (CP) cistron. The entire CP cistron was amplified using polyvalent primers for all PVY groups and amplified a 1159 nucleotide fragment. Two restriction endonucleases (HaeIII and MvaI) were used for RFLP analyses of RT-PCR products were chosen based on genomic sequences available for PVY group C, which includes almost all pepper isolates. RT-PCR-RFLP analyses revealed between two and four DNA profiles. Consequently, only 85 of the 167 PVY positive samples from Hatay were analysed for CP genome segment and they all had the diversity. None of the samples from all other localities had the diversity.

The impact of plant pathogens on post-weed biocontrol restoration

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Phytopathology 101:S24

Successful biological control of the deep-rooted perennial invasive plant Euphorbia esula/virgata, has dramatically reduced stand density at several locations within Theodore Roosevelt National Park in North Dakota, U. S. A. The synergistic interaction of root-feeding larvae of Aphidina spp. with soilborne plant pathogens has been shown to be the essential mechanism through which infestations of E. esula/virgata were controlled. We investigated the hypothesis that the biotic legacy from biocontrol would be transmitted to a soilborne disease on native species transplanted into restoration plots at sites where E. esula/virgata is under successful biocontrol. Seedlings of several native forbs and grasses were transplanted into plots at five different locations within the park where E. esula/virgata had been controlled. Surveys of the plots were conducted. Transplanted seedlings had a low disease incidence based on isolations from roots and crowns of sampled plants. Native forbs showed the most apparent disease; among these, the native species Ratibida columnifera, Aster ericoides, and Helianthus pauciflorus had the highest incidence of root and crown disease from which Rhizoctonia, Fusarium and Pythium spp. were isolated. Insect/pathogen interaction-driven stimulation of soilborne pathogen inoculum potential may affect the success of restoration following biological control, particularly for forbs. The results of pathogenicity tests on native forbs will be reported.

Dissecting the mode of transmission of Triticum mosaic virus and Wheat streak mosaic virus exacerbates loss of fresh and dry matter

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Phytopathology 101:S24

Wheat streak mosaic virus (WSMV) and Triticum mosaic virus (TriMV) are common leaf mosaics in the Great Plains of the United States. Information on TriMV’s effect on yield is limited. The current study evaluated the combined effects of TriMV and WSMV on yield and disease symptoms in a field experiment conducted in eastern Montana. In TriMV- and WSMV-inoculated plants, SFW was reduced by 64.1% in Millennium or Mace, but was significantly (LSD, P = 0.05) lower only in TriMV + WSMV-inoculated Millennium (WSMV-susceptible) and Mace (WSMV-resistant) in both cultivars. RFW and RDW did not differ between TriMV- and WSMV-inoculated plants in Millennium or Mace. The combined effects of TriMV + WSMV-inoculated plants, SFW was significantly reduced only in Millennium. SFW and RDW were different between TriMV- and WSMV-inoculated plants in both cultivars. RFW and RDW did not differ between TriMV- and WSMV-inoculated plants in Millennium or Mace, but were significantly lower in inoculated than in non-inoculated Millennium plants. In TriMV + WSMV-inoculated plants, SFW was reduced by 64.1% in Millennium and 11.3% in Mace; SDW was reduced by 47.3% in Millennium and 3.5% in Mace; RFW was reduced by 74.5% in Millennium and 15.5% in Mace; and RDW was reduced by 79.3% in Millennium and 29.5% in Mace. These results imply a high risk for yield loss when susceptible wheat is co-infected with TriMV and WSMV.

Dissecting the mode of transmission of Maize chlorotic mottle virus by the corn thrips, Frankliniella williamsi

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Phytopathology 101:S24

Maize chlorotic mottle virus (MCMV, Machlomovirus, Tombusviridae) has been recorded in Hawaii since the early 1990’s and has since become one of the most widespread viruses affecting corn production on the Islands of Kauai and Oahu. MCMV is transmitted semi-per sistently by several Chrysomelidae beetles including the western corn rootworm, Diabrotica virgifera (Coleoptera). However, beetles capable of transmitting the virus are not present in Hawaii where the main vector has been reported as the corn thrips, Frankliniella williamsi (Thysanoptera, Thripidae). We have examined the mode of transmission of MCMV by the corn thrips by using leaf disk assays. Adults of the corn thrips transmitted the virus right after acquisition, with no evidence for latent periods. Thrips were able to transmit the virus for up to 6 days after acquisition, with decreasing efficiency as time progressed. Transmission efficiency was higher when the thrips were allowed to feed on infected leaves for longer periods. Thrips were able to acquire the virus from infected plants and to transmit the virus to healthy leaves of different varieties of corn. Transmission rate peaked to 82% of inoculated leaf disks after an Acquisition Access Period of 48 hours and an Inoculation Access Period of 96 hours; 3 hours were estimated as the minimum time for virus acquisition and inoculation. MCMV was transmissible by both larvae and adults; however, adults were more efficient vectors than larvae. Our data suggests that corn thrips transmits MCMV in a semi-per sistent manner.
Genome sequencing and analysis of Anisogramma anomala, the causal agent of eastern filbert blight
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Phytopathology 101:S25

Eastern filbert blight (EFB), caused by the obligate filamentous ascomycete Anisogramma anomala (Peck) E. Müller, is a devastating disease of European hazelnut, Corylus avellana. The causal fungus is native to a wide area east of the Rocky Mountains, where it is found associated with its natural host, C. americana. Despite quarantine efforts, EFB was discovered in Washington in the late 1960s and now threatens the U.S. hazelnut industry, located primarily in the Willamette Valley of Oregon. Commercial cultivars carrying a single dominant resistance gene from ‘Gasaway’ have shown complete resistance to A. anomala infection over several decades of exposure in the west coast where, due probably to a single point introduction, the fungus has limited diversity. However, isolates from the east coast have been shown to overcome the ‘Gasaway’ gene, suggesting greater diversity across its natural range. To explore the pathogen genome for mechanisms of pathogenicity and look for molecular markers to trace pathogen diversity and movement, we have sequenced the genome of A. anomala using the Illumina GA IX platform. Approximately 24M 151x2 paired-end reads were generated from an insert library of ~400 bp. Initial de novo genome assembly achieved contig N₅₀ of 1.6 kb and N₉ₐ₀ of 20 kb. We are using a small number of high-quality Sanger sequences to validate and guide the improvement of the assembly, which will be followed by scaffolding. Genome assembly, annotation and analysis will be presented.

Genome-wide identification and characterization of microsatellite markers in Anisogramma anomala
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Phytopathology 101:S25

Anisogramma anomala (Peck) E. Müller is an ascomycete that causes eastern filbert blight (EFB) of European hazelnut, Corylus avellana. In the Willamette Valley of Oregon, where 99% of the U.S. crop is produced, EFB is controlled by fungicide applications and more recently through resistant cultivars carrying a single dominant resistance gene from ‘Gasaway’. The pathogen appeared in the Willamette Valley only after the 1960s. Harborred by its natural host, C. americana, it is believed to have a more diverse population east of the Rocky Mountains than in Oregon. Supporting this premise, some isolates from the east have been shown to overcome the ‘Gasaway’ resistance gene in field and greenhouse studies. To develop molecular markers for investigation of its genetic diversity and movement, we screened for perfect microsatellites and compound microsatellite sequences in a draft genome assembly of A. anomala. A total of 44,530 microsatellites were identified, with 134 identified per Megabase of genome. Compound microsatellites were found in 2157 loci. PCR primers were designed to amplify each microsatellite. Clustering analysis was being conducted to identify redundant loci. We assembled a collection of A. anomala isolates from diverse locales spanning its native range, including those expressing differences in virulence on ‘Gasaway’. Isolates were cultured, DNA was extracted, and we are now using them to screen non-redundant microsatellite markers with motifs of two or more nucleotides.

Co-infection of a single Phytophthora infestans isolate by two distinct viruses
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Phytopathology 101:S25

Phytophthora infestans is the highly destructive pathogen that causes potato and tomato late blight. It has fungal-like morphology and habitat, but belongs to a distinct group, the oomycetes. Screening of P. infestans isolates revealed two double-stranded (ds) RNAs, 8.2 kb and 3.0 kb, in a single isolate from Florida. The dsRNAs represent genomes of two distinct viruses, as evidenced from their complete sequences and biological properties. The 8.2 kb dsRNA, named Phytophthora infestans RNA virus 3 (PIRV-3), has two open reading frames (ORFs) showing closest similarity to the respective ORFs in Phlebiopsis gigantea mycovirus dsRNA2 and Fusarium graminearum dsRNA mycovirus-3, two viruses with similarity to members of the family Togaviridae. ORF1 and ORF2 are in different frames on the same strand with a small overlap, but no translational frameshifting signal was detected. The 3.0 kb dsRNA, named Phytophthora infestans RNA virus 4 (PIRV-4), contains a single ORF predicted to encode a protein with greatest similarity to the RNA-dependent RNA polymerases of Saccharomyces cerevisiae 20s and 23s viruses, two members of the family Narnaviridae. PIRV-4 was also detected in isolates without PIRV-3. A combination of chemotherapy and hypothyroidism cured PIRV-3 from the host plant, resulting in a PIRV-3-free strain with denser mycelium in culture.

Identification of a soybean G-protein coupled receptor and its role in plant defense responses
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Phytopathology 101:S25

G-protein coupled receptors (GPCR) comprise a large family of transmembrane receptors. After binding a ligand, the GPCR activates a cognate G-protein which, in turn will trigger a myriad of second messengers that regulate numerous cell physiological responses. Recent studies in plants have demonstrated direct roles for G-proteins in plant defense responses including the production of reactive oxygen species (ROS), the activation of NADPH oxidases, ion channels and phospholipases. A heterotrimeric G-protein in Sclerotinia sclerotiorum (an ascomycete) has been demonstrated to play a role in the jasmonate-mediated signaling response to the necrotrophic pathogen Alternaria brassicola. Based on our previous microarray studies we have identified several components of the G-protein signaling cascade that consistently change in expression during pathogenesis. To further assess if there is an actual GPCR involved directly or indirectly in the interaction between the necrotrophic fungal pathogen Sclerotinia sclerotiorum and soybean, we used the Arabidopsis GCR1 sequence to screen the soybean genome for candidate GPCR coding genes. Two putative GPCR genes were identified. An RNAi silencing construct was generated with the sequence and was introduced into soybean. We are currently in the process of obtaining viable seeds from the silenced plants. Additionally, Arabidopsis gcr1 mutants are being evaluated for their response to Sclerotinia sclerotiorum and other pathogens.

Novel rust resistance in wheat (Triticum aestivum L.)
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Phytopathology 101:S25

The Puccinia fungi that cause wheat rust diseases are among the most globally destructive agricultural pathogens. The most effective and utilized defense against rust is genetic resistance. The vast majority of rust resistance is race-specific conferred by single genes rapidly overcome by the pathogens. From EMS mutagenesis using a soft white spring wheat cultivar ‘Alpowa’, we have identified a mutant, MNs220 Alp has enhanced resistance to leaf, stem, and other rust races. Genetic analysis in several backcrosses confirms that the resistance found in MNs220 Alp is conferred by a single dominant gene. Gene expression profiling of several pathogenesis-related (PR) genes indicates that MNs220 Alp2 has a rapid and elevated pathogen induced response. The mutation is likely to be associated with the change of negative regulation of resistance that is known to suppress either active or passive defense responses. In wheat, negative regulators have been observed to inhibit rust resistance genes. MNs220 Alp has enhanced resistance to leaf, stem (including Ug99 and its derivative races), and stripe rust, as well as a few races of leaf rust. Genetic analysis in several backcrosses demonstrates that the resistance found in MNs220 Alp is conferred by a single dominant gene. Gene expression profiling of several pathogenesis-related (PR) genes indicates that MNs220 Alp has a rapid and elevated pathogen induced response. The mutant has an indistinguishable phenotype from the wild type in the absence of pathogens allowing for the immediate deployment into breeding programs. Beyond the immediate benefit, continued analysis of the locus will lead to a better understanding of the regulation of defense response network in wheat.

Effects of plant growth regulators on aDMI insensitive Sclerotinia homoeocarpa population
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Dollar spot (caused by Sclerotinia homoeocarpa) is primarily controlled by fungicide applications on golf courses. Demethylation inhibitor fungicides (DMIs) are the most widely used of the three fungicide classes with confirmed resistance in dollar spot. Plant growth regulators (PGRs; flurprimidol and paclobutrazol), which inhibit gibberellic acid, have a correlation in vitro sensitivity to DMIs and exhibit a fungistatic effect on dollar spot in the field. There is no report of PGRs selecting DMI insensitive isolates in the field. The objective of this study was to evaluate the effect of PGRs on golf course populations of S. homoeocarpa consisting of both DMI sensitive and insensitive isolates. The effects of the PGRs in rotation and tank-mixed with the DMI, procipazole were studied to determine changes in efficacy of dollar spot infection centers similar to propiconazole alone at 2 oz/1,000 ft². These treatments also selected for DMI insensitive isolates 7 days after application.
Wheat powdery mildew is an important disease of wheat in China. The field experiments were conducted at the experimental station of CAAS in Hebei Province in 2007, 2008 and 2009. Two wheat varieties were used in the experiments and different epidemic patterns of powdery mildew were obtained by fungicide spraying. The canopy reflectance of wheat with different disease intensity was measured by using spectroradiometer. The results indicated that as disease index increasing, reflectance in the near infrared region decreased significantly, and there were highly significant negative correlations between them in both varieties at the anthesis and milk-filling stages. Meanwhile the first derivative reflectance in visible region and in near infrared region were positive and negative correlations with disease index, respectively. Red edge position moved to short wavelength when wheat powdery mildew started to develop within 10 days and X. campestris was re-isolated and identified using the above methods. Xanthomonas campestris has been previously reported on other species of Ficus in Florida. Further characterization of the pathogen, host range studies, and the effect of temperature and light on disease development are underway.

A sensitive molecular method for detecting virus in orchids
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Cymbidium mosaic virus (CymMV) and Odontoglossum ringspot virus (ORSV) are the most prevalent orchid viruses in nursery production worldwide. These two economically important viruses are a threat to the industry as they cause size reduction and decrease foliar and flower quality in numerous orchid species. Virus symptoms in orchids are typically not distinct and often asymptomatic, making visual detection unreliable. Common virus detection methods include immunological (i.e. double antibody sandwich (DAS-ELISA) and Immunostrips) nucleic acid hybridization (i.e. dot-blot) and molecular assays (i.e. PCR and real time PCR). In this study, high-fidelity and standard RT-PCRs were used to detect CymMV and ORSV from infected orchid leaf tissue. The high-fidelity RT-PCR detected 0.001 ng/µl of CymMV and ORSV in a total RNA extraction compared to 1 ng/µl of CymMV and 0.1 ng/µl of ORSV respectively. These results indicate a 10³ increase in sensitivity for detecting CymMV and ORSV using the high-fidelity RT-PCR. We present a highly sensitive method that can be used to ensure virus free orchids that meet phytosanitary requirements. For this purpose a diagnostic manual with detailed methodologies is in progress.

Increases in snap bean and soybean seedling diseases associated with a chloride salt and changes in the micro-partitioning of root tap cement
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Phytopathology 101:S26
In a series of field experiments from 1995 through 2010, the incidence of seedling diseases of snap bean and soybean caused by Rhizoctonia solani, Macrophomina phaseolina, Puccinia spp., and Fusarium spp. were greater with an application of KCl than with K₂SO₄ applied at 93 kg K/ha. To determine if the observed increases could be due to a change in root calcium associated with chloride salts, soybeans were grown in pasteurized silt loam controls. Plants were placed in a greenhouse and shade house where temperature ranged from 23–32 C and 60–95% relative humidity. Symptoms started to develop within 10 days and X. campestris was re-isolated and identified using the above methods. Xanthomonas campestris has been previously reported on other species of Ficus in Florida. Further characterization of the pathogen, host range studies, and the effect of temperature and light on disease development are underway.

Plasmid content of Erwinia amylovora isolates from orchards in Washington and Oregon
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Phytopathology 101:S26
We examined the plasmid content of a collection of 305 isolates of Erwinia amylovora from Washington and Oregon in the Pacific Northwest of the U.S.A. with PCR assays and RFLP. Nearly all isolates of E. amylovora carried plasmid pEA29, which is not found in other species of bacteria, but 4% of the isolates from this region lacked pEA29. The plasmid pEU30, previously reported in pathogenic strains from western states in the U.S.A., was detected in 28% of isolates. The RFLP patterns of plasmid preparations from a third of isolates from an epidemic in Washington in 1988 had altered RFLP patterns, possibly due to the presence of plasmid(s) in addition to pEA29 or pEU30. Considering all samples, the majority of isolates in this region were typical of E. amylovora and harbored only pEA29. Nonetheless, many of the pathogen isolates had altered plasmid content, indicating that plasmid acquisition and propagation in populations of E. amylovora in orchards in the Pacific Northwest is more common than previously assumed.

Epidemiology of grape anthracnose: Identification of factors associated with defoliation of grape leaves infected by Elsinoe ampelina
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Phytopathology 101:S26
Anthracnose is a serious disease on several winter hardy grape cultivars. Infected leaves drop prematurely and severe epidemics may result in poor or
Efficacy of seed treatments on resistant bacterial strains and reduce copper build-up in soils. Treatments failed to prevent infections and provided less than 19% reduction.

Management of strawberry anthracnose fruit rot in North Carolina with reduced fungicide spray schedules

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Phytopathology 101:S27

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Effect of alternative products on mortality of adults of citrus black fly Aleurocanthus woglumi Ashby, 1915 on leaves of orange trees

R. A. CARVALHO (1)

Phytopathology 101:S27

Effect of alternative products on mortality of adults of citrus black fly Aleurocanthus woglumi Ashby, 1915 on leaves of orange trees

R. A. CARVALHO (1)

Phytopathology 101:S27

To determine the effect of Thielaviopsis basicola on soybean, a greenhouse disease screening trial was conducted to evaluate the susceptibility of popular varieties grown in Arkansas and determine the efficacy of seed treatments. Since, Thielaviopsis basicola is considered a cool wet weather pathogen; a temperature controlled soil bed was constructed using a chiller that circulates water through copper coil tubing in the soil to maintain soil temperatures in the mid-50 o F. The soil was autoclaved and artificially inoculated with T. basicola at ~100 propagules/gram of soil. Four Delta Grow soybean varieties (5750, 4970, 4880, 4975, and 5160) were treated with four different seed treatments (Untreated, Experimental Treatment 1, Apron Max, Apron Max + Experimental Treatment 1) and planted in the inoculated soil in a randomized complete block design. Growing conditions for the soybeans were 14 hour days at 86°F air temperature for 35 consecutive days. On the 35th day, the roots were washed for 20 minutes. They were then surface sterilized in 10% bleach solution for one minute and 30 seconds. The roots were plated on a TBCEN selective media. Initial results indicated all soybean varieties tested were susceptible to Thielaviopsis basicola. After only two week after planting, untreated plant roots were discolor and found to have T. basicola chlamydospores present on damaged tissue.

Alternative control of citrus black fly Aleurocanthus woglumi Ashby, 1915 in the northeast of Brazil

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Phytopathology 101:S27

Citrus black fly Aleurocanthus woglumi is an invasive pest in Brazil and is restricted to a few states. Its detection in the northeastern states in 2010 caused an interruption of exportation of fruits from the attacked area, originating huge losses. Local government has urged the producers to spray their crops with imidacloprid which is the only product registered in Brazil for its control. This action has displeased the organic producers who were left without options. So, this study had the objective of testing alternative products on the control of citrus black fly. Mineral oil, vegetable oil, neem oil, orange peel oil, detergent and soaps, at concentrations of 1.0% and 0.5%, were sprayed weekly over adults, eggs and larvae of citrus black fly. In the field, in a completely randomized design with 14 treatments and 10 replicates. Infested leaves were checked under stereo microscope. The efficiency of alternative products varied according to each phase of development. No alternative product was effective against hatching of eggs. Detergent, orange peel oil, mineral oil and powdered soap killed 100% of larvae of 2nd and 3rd instars with just one application. Only powdered soap and orange peel oil killed 100% of pupas. Both detergent and orange peel oil killed 100% of adults instantly. Thus, these products can be used as an alternative approach on the control of citrus black fly.
down and sprayed with 0.5 L garden sprayer containing water based solutions of detergent at the following concentrations: 5.0%, 4.0%, 3.0%, 2.0%, 1.0%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1% and 0.0% (water only - control) in a completely randomized design with 11 treatments and 10 replicates. Each replicate consisted of one infested leaf. From each sprayed branch, 10 leaves were collected and checked under stereo microscope. No live flies were found on leaves sprayed at concentrations ranging from 2.0% down to 0.2%. From 0.1% down to 0.04% both dead and live larvae were found on leaves treated at 0.2% and 0.1% concentrations in a completely randomized design with 5 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each sprayed leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. No live flies were found on leaves sprayed at concentrations ranging from 2.0% down to 0.2%. From 0.1% down to 0.04% both dead and live larvae were found on leaves treated at 0.2% and 0.1% concentrations in a completely randomized design with 5 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each sprayed leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. Detergent, powdered soap and orange peel oil killed 100% of flies. Mineral oil, vegetal oil, neem oil, orange peel oil, detergent and powdered soap left no live 2nd and 3rd instar larvae of citrus black fly on treated leaves after both 1 and 2 applications, being effective alternatives for controlling this pest at these phases.

Effect of concentrations of orange peel oil on mortality of adults of citrus black fly Aleurocanthus woglumi Ashby, 1915 on leaves of orange trees

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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Some alternative products sprayed at a concentration of 1.0% over slightly infested leaves of tangerine trees. Infested leaves were carefully cut, turned upside down and sprayed from a distance of 10 cm with a 20 L manual costal sprayer containing solutions of detergent at the following concentrations: 5.0%, 4.0%, 3.0%, 2.0%, 1.0%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1% and 0.0% (water only - control) in a completely randomized design with 11 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each sprayed leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. No live flies were found on leaves sprayed at concentrations ranging from 2.0% down to 0.2%. From 0.1% down to 0.04% both dead and live larvae were found on leaves treated at 0.2% and 0.1% concentrations in a completely randomized design with 5 treatments and 10 replicates. Each replicate consisted of one infested leaf. Flies were counted before and after each spraying. A cloth was put behind each sprayed leaf to capture the flies forced out by spraying. Leaves and cloth were checked under stereo microscope. The concentrations of 0.5%, 0.4%, 0.3% and 0.2% killed 100% of flies. At 0.1% concentration, the number of dead flies did not match the number of live flies, indicating that some have managed to escape. Thus, orange peel oil is 100% efficient at concentrations as low as 0.2% on the control of adults of citrus black fly.

Effect of alternative products on mortality of 2nd and 3rd instar larvae of citrus black fly Aleurocanthus woglumi on leaves of lemon trees

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Phytopathology 101:S28

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Orange peel oil has been found to control 2nd and 3rd instar larvae of citrus black fly on leaves of orange trees at 1.0% and 0.5% concentrations. This study had the objective of testing the effect of other concentrations of orange peel oil sprayed over infested leaves of orange trees. Highly infested newly developed branches were carefully cut, turned upside down and sprayed with a 0.5 L garden sprayer containing water based solutions of orange peel oil at the following concentrations: 2.0%, 1.0%, 0.8%, 0.6%, 0.4%, 0.2%, 0.1%, 0.08%, 0.06%, 0.04%, 0.02% and 0.0% (water only - control) in a completely randomized design with 12 treatments and 10 replicates. Each replicate consisted of one infested leaf. From each sprayed leaf, 10 leaves were collected and checked under stereo microscope. No live larvae were found on leaves sprayed at concentrations ranging from 2.0% down to 0.2%. From 0.1% down to 0.04% both dead and live larvae were found. No dead larvae were found at 0.02% concentration nor on the control treatment. Thus, since it is effective at even very low concentrations, orange peel oil can be safely used on the alternative control of adults of citrus black fly.
Effect of alternative products on mortality of 4th instar larvae (pupas) of citrus black fly Aleurocanthus woglumi on leaves of orange trees

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Phytopathology 101:S29

Citrus black fly is an invasive species in Brazil and is restricted to a few states. The only product registered in Brazil for its control is imidacloprid, but its utilization has been avoided by ecological farmers. Some alternative products have been found to be effective against adults of citrus black fly. This study had the objective of testing the effect of alternative products on mortality of the 4th instar larvae (pupas). Powdered soap, mineral oil, vegetal oil, and orange peel oil were tested at 1.0% and 0.5% concentrations. Detergent was tested at 5.0% and 2.5% concentrations. High infested leaves received three weekly applications with a 0.5 L garden sprayer from a distance of 10 cm. Treated and control leaves were protected with a cloth in the field. From each treatment, 4 leaves were collected 7 days after the first application, 10 leaves were collected 7 days after the second application and more 10 leaves were collected 15 days after the third weekly application. Treated leaves were checked under stereo microscope. A needle was used to perforate each pupa in search for organic fluids. Powdered soap killed 100% of pupas only at 1.0% concentration since the first application. Orange peel oil killed 100% of pupas at both 1.0% and 0.5% concentrations. None of the alternative products tested in this study killed 100% of pupas of citrus black fly.

Multilocus analysis of Phoma sclerotioides isolates from Minnesota

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Phytopathology 101:S29

Phoma sclerotioides causes brown rot in alfalfa, which reduces winter survival. Regional genotypes occur but local population structures have not been characterized. The population genetic structure and gene flow within alfalfa fields in Minnesota was inferred using multilocus analysis. Portions of chitin synthase 1 (CHS; 299 bp), glyceraldehyde-3-phosphate dehydrogenase (G3PDH; 578 bp), the rDNA internal transcribed spacers (ITS; 498 bp), and their concatenated sequences (CS) (1,375 bp), were analyzed for 102 isolates from four sites. Diversity of the CS was similar for two collection years with regard to nucleotide (?) and haplotype (Hd) diversity, as well as Theta (?) values, although a different number of haplotypes were found each year. Sequence of G3PDH was the most diverse for ? and ? values among other estimators, while CHS had the highest Hd in both years. In 2007, no population differentiation was found between isolates from two sites with the CS, and abundant gene flow (Nm ? 31.50) was detected. In 2008, highly significant population differentiation between isolates from four sites, including the two sampled in 2007, and more restricted gene flow (Nm = 3.15) was detected. Neutrality tests on the individual or joint sequences were not statistically significant. The level of nucleotide and haplotype diversity indicates that the Minnesota population is not recently introduced and nucleotide variation is mostly driven by random genetic drift.

Assembling and exploring the Cochliobolus miyabeanae genome of a strain pathogenic on wildrice (Zizania palustris)

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Phytopathology 101:S29

The genome of a strain of C. miyabeanae was shotgun sequenced by paired-end reads with Illumina HiSeq 2000 technology. The genome was assembled with Abyss software yielding a total size of 34.96 Mb (114X), with N50 = 99.43 kb contained in the largest 105 scaffolds, and with a maximum scaffold length of 408.932 kb. The G + C content of the genome was 51%. GeneMark-ES v2.3a detected 12,344 protein-coding genes, with a mean size of 1,537 bp and an average of 2.55 exons per gene. The average size of encoded proteins was 437 aa. Annotation of fungal proteins was done using BLASTP against the protein database of NCBI and protein functional classification was done with Blast2GO. Complete genome blast searches to the pathogen-host interaction database identified 15% of the genes mostly related to virulence, and pathogenicity, and a few effector molecules. Additionally, several genes were associated with transport, resistance and sensitivity to chemicals. The most abundant repetitive elements identified belong to the LTR-Gypsy retrotransposons. An independent RNA sequencing experiment is being used to validate the genome assembly. DNA genomic sequences from C. miyabeanae were mapped onto a reference genome (C. heterostrophus). Understanding the organization of the C. miyabeanae genome in parallel with the fungal transcriptome can help in developing wildrice varieties that will resist the specific pathogenicity/virulence factors of the fungus.

Evaluation of Bacillus firmus strain GB-126 seed treatment for the biocontrol of the reniform nematode on cotton plants

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Phytopathology 101:S29

Previously we have demonstrated the biocontrol potential of Bacillus firmus strain GB-126 in a non competitive autoclaved soil environment. Bacillus firmus strain GB-126 reduced numbers of Rhyzoplunka reniformis females, eggs and eggs per gram of cotton root at a rate of 0.71 mg of spores per seed. The objective of this study was to evaluate the seed formulation rates of 0.10, 0.71, and 1.40 mg of spores per seed of B. firmus strain GB-126 on cotton compared to albicarb and an untreated control in a silt loam field soil under greenhouse conditions. Variables measured every 5 days were plant height, shoot and root weight, root architecture, females and eggs per gram of root, and verminful per 480 grams of soil. The highest seed treatment rate of 1.40 mg of spores of B. firmus strain GB-126 per seed provided protection to the cotton root from 20 DAP until 30 DAP where the number of females per gram of root were lower than the untreated seed treatment (P ≤ 0.027), and resulted in lower numbers of eggs per gram of root 30 DAP (P ≤ 0.001). The three seed treatment rates of B. firmus strain GB-126 reduced verminful populations in soil 15 DAP (P ≤ 0.001) with the highest rate suppressing the verminful population through 30 DAP (P ≤ 0.005). The 1.40 mg of spores per seed rate reduced numbers of the females, eggs, and verminful life stages of R. reniformis 30 DAP, similarly to albicarb (P ≤ 0.05).

Occurrence of a soft-rot disease on Oncidium orchids caused by a Dickeya sp. in Florida

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Phytopathology 101:S29

Oncidium orchids have been subjected to extensive cultivation in the pot-plant and cut flower industries. In August 2008, approximately 50 Oncidium ‘Gower Ramsey’ orchids were discovered at a commercial orchid nursery in South Florida with brown, macerated leaves and pseudobulbs typical of soft-rot disease reported in other orchids. Ten plants were selected and sections were removed from the edge of symptomatic tissue. All isolates were Gram negative, anaerobic, degraded pectate, grew at 37°C, produced blue to brown pigment on NGM medium, were sensitive to erythromycin, were oxidase negative, and were positive for phosphatase and indole production. MIDI analysis (Sherlock version TSBA 4.10; Microbial Identification, Newark, DE) identified the strains as Erwinia chrysanthemi (SIM 0.880 to 0.929). PCRs were performed using the 16S primers 27f and 1495r and 1,423 bp of the 16S rDNA gene showed 98 to 99% similarity to Pectobacterium chrysanthemi. Pathogenicity tests were performed by injecting 10 Oncidium ‘Gower Ramsey’ orchids with 100 µl of a 1 × 10^8 CFU/ml of a bacterial suspension. A Dickeya sp. was re-isolated and identified according to the method described above. Although an Erwinia sp. has been reported to cause soft-rot symptoms on Oncidium aureum, to our knowledge this is the first report of a Dickeya sp. (=E. chrysanthemi) causing soft-rot symptoms on an economically important Oncidium orchid in large-scale production in the Florida.

Expression of the cloned IS33 transposase promoter from Pseudomonas savastanoi under stress conditions

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Phytopathology 101:S29

Pseudomonas savastanoi causes tumors on olive and oleander trees, in part by the synthesis of indole-3-acetic acid (IAA) and cytokinins (CK). Oleander isolates carry the IAA and CK genes on virulence plasmids which also contain IS elements that have been associated with loss of virulence due to deletion of the IAA genes. The role of IS elements in bacterial mutation is well-established, but it is not known if mutation rates, at least those mediated by IS transposition events, can change in response to environmental stresses. The transposase (tp) gene of the P. savastanoi IS33 is homologous to heat-shock promoter sequences from E. coli. Thus, the tp gene of IS33 may be upregulated by heat-shock or other environmental stresses. To test this hypothesis, IS33 was cloned into a TOPO TA cloning vector, and the putative promoter (tpnp) was subcloned into pH0155 upstream of a promoterless GS5 (uidA) reporter gene. If tpnp is a heat-shock promoter, then under different temperature conditions, there should be different levels of expression of the tpnp/uidA fusion. The purpose of this masters’ research is to measure tnp activity under stress conditions, and to identify and quantify the movement of transposable genetic elements (TGEs) in P. savastanoi under different stress conditions as a potential mechanism for environmentally-regulated mutation.
Diversity in Cotton leaf curl virus (CLCuV) isolates prevalent in northwestern India in light of the breakdown of CLCuV resistance in cotton

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Phytopathology 101:S30

Following the reports of breakdown of Cotton leaf curl virus (CICuV) resistance in popular cotton hybrids in northwestern India during the 2010 season, six strains of CLCuV, including Sri Ganganagar strain isolated from a severely infected CLCuV-resistant hybrid, were characterized and nucleotide sequence alignments with T DNA components were determined. Sequence comparisons revealed 81–99% and 88.3–92% sequence identity of DNA-A and βDNA, respectively, with known CLCuV sequences. Recombination analysis revealed significant recombination in these six virulent Indian strains showing 25 recombination sites in DNA-A and 11 recombination sites in βDNA. The observed recombination in several regions of DNA-A and βDNA in the potential resistance breaking Sri Ganganagar strain of CLCuV was mapped to the highly virulent Burewala strain and several other strains.

Characterization of the ICIRSAT mini-core peanut germplasm collection regarding Sclerotinia blight resistance and oleic acid composition

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Phytopathology 101:S30

Peanut production is consistently threatened by various diseases and pests. Sclerotinia minor Janss (R. hortorum), the causal agent of Sclerotinia blight, is a major threat to peanut production in the Southwestern U.S., Virginia, and North Carolina and can reduce yield by up to 50% in severely infested fields. Although host plant resistance would provide the most effective solution to managing Sclerotinia blight, limited sources of resistance to the disease are available for use in breeding programs. Peanut germplasm collections are available for exploration and identification of new sources of resistance, but traditionally the process is lengthy, requiring years of field testing before those potential sources can be identified. Molecular markers associated with phenotypic traits can speed up the screening of germplasm accessions. This study objective of this study was to characterize the ICIRSAT mini-core collection with regards to oleic acid composition and a molecular marker associated with Sclerotinia blight resistance. One hundred twenty-four (124) accessions from the collection were available and genotyped using the SSR marker and 67 were identified as potential new sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance. Capillary electrophoresis profiles of oil extracted from each accession determined that none were high oleic in composition.

The effect of biological control practices on inducible defense genes and metabolic genes in field-cultivated potato plants

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Phytopathology 101:S30

Potato production is consistently threatened by various diseases and pests. The causal agent of Sclerotinia blight, is a major threat to potato production in the Southwestern U.S., Virginia, and North Carolina and can reduce yield by up to 50% in severely infested fields. Although host plant resistance would provide the most effective solution to managing Sclerotinia blight, limited sources of resistance to the disease are available for use in breeding programs. Peanut germplasm collections are available for exploration and identification of new sources of resistance, but traditionally the process is lengthy, requiring years of field testing before those potential sources can be identified. Molecular markers associated with phenotypic traits can speed up the screening of germplasm accessions. This study objective of this study was to characterize the ICIRSAT mini-core collection with regards to oleic acid composition and a molecular marker associated with Sclerotinia blight resistance. One hundred twenty-four (124) accessions from the collection were available and genotyped using the SSR marker and 67 were identified as potential new sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance. Capillary electrophoresis profiles of oil extracted from each accession determined that none were high oleic in composition.

Identification of the critical factors for mechanical transmissibility of Tomato leaf curl New Delhi virus

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Phytopathology 101:S30

Tomato leaf curl New Delhi virus (ToLCNDV) belonging to Begomovirus was identified from many plants in tropical and subtropical countries. ToLCNDV contains bipartite genome, designated as DNA-A and DNA-B and each is approximately 2.7 kb in size. In April 2007, a new isolate ToLCNDV-OM was isolated from oriental melon (Cucumis melo). This isolate could infect some important gourd crops like cucumber, luffa and zucchini. Interestingly, many begomoviruses cannot be mechanically transmitted to their original hosts. However, ToLCNDV-OM could be transmitted to its original host by mechanical inoculation. In order to investigate the factors affect the mechanical transmissibility of ToLCNDV, the DNA-A and DNA-B of ToLCNDV-OM were recombined with the genome of a mechanically non-transmissible ToLCNDV cucumber isolate (ToLCNDV-CB). The virus inocula were prepared from infected Nicotiana benthamiana by agro-infiltration of clones and then these mechanical transmitting clones were introduced into oriental melon and tomato plants to evaluate the mechanical transmissibility of the virus. Results indicated that the combination of DNA-A of ToLCNDV-CB with DNA-B of ToLCNDV-OM became mechanically transmissible whereas that of DNA-A of ToLCNDV-OM with DNA-B of ToLCNDV-CB did not. It clearly suggested that DNA-B of ToLCNDV-OM contain critical factors for mechanical transmissibility. Study for identification of specific gene that is associated with mechanical transmissibility of ToLCNDV-OM is ongoing and will be presented.

Functional characterization of two genes involved in cercosporin biosynthesis in Cercospora kikuchii

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Phytopathology 101:S30

Cercospora kikuchii is the causal agent of soybean leaf blight and purple seed stain diseases. Currently, leaf blight is a major concern in Louisiana and other southern states, and it has the potential of spreading into other major soybean producing regions, such as the Midwest. The pathogen produces a toxin, cercosporin, which was shown to play a crucial role in pathogenicity and virulence on soybeans. We utilized two-dimensional protein gel electrophoresis (2DGE) to identify proteins that may be involved in cercosporin biosynthesis by comparing protein profiles of C. kikuchii grown under cercosporin-favoring (light) and cercosporin-suppressive (dark) conditions. Several proteins were up-regulated in C. kikuchii grown under light, and these were sequenced and identified as hydroxynaphthalene reductase (HNR) and adenosylhomocysteinase (AHC). The corresponding genes were cloned from C. kikuchii through genome walking. HNR gene is predicted to be 862 bp long with one intron, whereas AHC gene is predicted to be 1562 bp long with two introns. HNR and AHC gene disruption mutants were produced through a hygromycin split marker approach, and the mutants showed drastic reduction in cercosporin production in vitro. The resulting HNR and AHC mutants also are being tested for changes in pathogenicity or virulence on soybean.

Fungicide seed treatments to manage seedling blight of faba bean in Alberta, Canada, 2010

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Phytopathology 101:S30

Faba bean (Vicia faba L.) is a source of high energy and protein for livestock and humans. Root rot of faba bean caused by Fusarium avenaceum and Rhizoctonia solani is widespread on the Canadian prairies. To examine the efficacy of four fungicides Apron Maxx (metalaxyl +fludioxonil), T-280 (carboxin + thiram), SP1020 and Trilex (trifloxystrobin + metalaxyl) against these pathogens, two inoculated field trials, each treated with one pathogen, were conducted at Lacombe, Alberta in 2010. Each trial consisted of the faba bean cultivars Earlibird and Snowbird, inoculum at 15, 30 or 35 mL/6-m row, the fungicides, in a randomized split plot design with cultivar as main plots and sub-plots, along with inoculated and non-inoculated controls. Seedling emergence and seed yield declined and root rot severity increased with increasing inoculum concentration for both pathogens. Diseased plants were shorter and had thinner stems than healthy plants and the lower foliage turned yellow. The cv. Snowbird showed more susceptibility compared to Earlibird. Seed from diseased plants was often shrunken. Each of the fungicides improved emergence and yield compared to the inoculated control. This indicates that seed treatment fungicides may be useful in reducing the impact of these ubiquitous pathogens on seedling establishment and yield.
Pathological and molecular race determinations of Fusarium oxysporum f. sp. lactucae from Taiwan
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Phytopathology 101:S31
Fusarium wilt disease of lettuce (Lactuca sativa L.), caused by the vascular wilt pathogen Fusarium oxysporum f. sp. lactucae (FoLa), is one of the major factors restricting stable production of lettuce in Taiwan. Identification of pathological races of FoLa is a necessity for lettuce breeders to develop FoLa-resistant cultivars and for growers to choose appropriate cultivars for production. For race determination, Japanese FoLa reference strains and FoLa isolates from Taiwan were subjected to pathogenicity test against three differential lettuce cultivars, Patriot, Costa Rica No. 4, and Banchu Red Fire. Results showed that most of the FoLa isolates from Taiwan were race 1, with the exception of two isolates (FoLa-10 and FoLa-40) collected from Taoyuan County were identified as race 3. This is the first report revealing that the FoLa race 3 was found outside of Japan. Based on random amplification of polymorphic DNA (RAPD) fingerprinting, genetic diversity in FoLa races was observed. Several RAPD markers were selectively used as schematics to differentiate FoLa races in Taiwan. (Supported by 95AS-13.3.1-BQ-B1(6), 96AS-14.3.1-BQ-B1(6), 97AS-14.3.1-BQ-B1(6), 99AS-9.3.1-BQ-B2(6), the ATU plan, Ministry of Education, Taiwan, R.O.C., and National Chung Hsing University, Taiwan, R.O.C.)
Phylogenetic relationship of Xylella fastidiosa between pear leaf scorch strains and strains of other host origins
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Phytopathology 101:S31
Pear leaf scorch, the only Xylella fastidiosa-induced disease reported from Taiwan, was found in area where the variety Hengshan (Pyrus pyrifolia) was grown. Strains of pear leaf scorch X. fastidiosa (XF-PLS) shared similarities to strains of other host origins in requirement of complex medium and rippled cell walls, however, recent serological and molecular biology studies showed difference among them. Four strains of XF-PLS were compared with 15 strains isolated from olive, pear, plum, peach, mulberry, grapes, citrus, coffee, and sycamore by sequence analyses of 16S rRNA and 16S-23S rRNA spacer region. When sequence analysis of 16S rRNA based on fragment size of 1537-1540 bp was compared, the similarity index among 4 XF-PLS strains was 99.8-99.9%, whereas that was 98.1-98.7% between XF-PLS strains and strains from other hosts. When sequence analysis of 16S-23S rRNA spacer region based on fragment size of 510-540 bp was compared, the similarity index among 4 XF-PLS strains was 100%, whereas that was 87.6-88.4% between XF-PLS strains and strains from other hosts. The phylogenetic trees revealed that XF-PLS strains were separated from strains of other hosts. Strains of other hosts were divided into four subgroups: strains from 1) oleander, 2) grape and mulberry, 3) citrus and coffee, and 4) pecan, peach, plum, and sycamore. Results indicate that XF-PLS strains were not closely related to the above-mentioned strains and could belong to a new subspecies of X. fastidiosa.
Monitoring Cercospora zeae-maydis sensitivity levels to quinone outside inhibitor fungicides across multiple years
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Phytopathology 101:S31
Gray leaf spot (GLS) of corn caused by Cercospora zeae-maydis, can cause serious losses to producers in the U.S. One GLS management practice used by some producers is the application of quinone outside inhibitor (QoI) fungicide tank mixes. A survey was conducted in the North Central U.S., which also revealed the means the risk of QoI sensitivity shifts may be higher in corn pathogens like C. zeae-maydis. The EC_50 sensitivity levels of C. zeae-maydis to QoI fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin) in isolates collected from different North Central states from 2008 to 2009 were compared with the sensitivity levels of baseline isolates. The mean azoxystrobin EC_50 level of isolates collected in 2008 and 2009 was similar to the baseline EC_50 level, but isolates collected in 2009 had a significantly greater pyraclostrobin EC_50 level compared to the mean of the baseline isolates. The mean trifloxystrobin EC_50 level of isolates collected in 2008, 2009, and 2010 were not significantly greater than the mean of the baseline isolates. These results indicate that sensitivity levels of C. zeae-maydis isolates to QoI fungicides can vary year to year, and that fungicide sensitivities should continue to be monitored.
Production of healthy seed potatoes on organic farms
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Phytopathology 101:S31
Organic growers face limited access to certified seed potatoes for specialty varieties. Our research will determine the feasibility of seed potato production on Wisconsin organic farms and aims to increase the number of varieties available. In 2010, trials on organic farms of potatoes grown from minitubers on raised beds covered with plastic mulch had yields of 40 lb/10 ft of row, which is approaching yields on conventional farms. Trials from 2007-2010 showed that Potato virus Y (PVY) incidence on organic farms was comparable to conventional seed potato farms. Winter wheat borders, live mulches, and mineral oil sprays were trialed as PVY control measures, but only mineral oil sprays provided virus control. Aphid landing data has not supported the hypothesis that aphids land on field edges, which could explain why winter wheat borders have been ineffective at controlling PVY. A diverse, tissue culture bank of 110 high priority varieties is being developed. In 2010, we conducted trials of 4 varieties each of red, yellow, russet and fingerling varieties on 4 organic farms. There were significant yield and size profile differences between varieties and locations. Varieties were tested for disease and leaf hopper resistance, nutritional attributes, and taste. Disease and leaf hopper resistance varied significantly across varieties. Economic analysis of selected varieties will include minituber production costs and potential profits for organic growers.
Occurrence of Northern stem canker in first soybean plantings following Conservation Reserve in South Dakota
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Phytopathology 101:S31
Northern stem canker, caused by Diaporthe phaseolorum var. caulivora (DPC), is a sporadic and unpredictable disease of soybeans in the North Central region. In 2009, a high level of disease (ca. 90% incidence) with severe yield loss (60–65%) was documented in a commercial field near Castlewood, South Dakota. Isolates from cankered soybean tissue showed typical DPC cultural characteristics and perithecia and are being confirmed by RFLP analysis of DNA amplified from the ITS region of rDNA. The remarkable feature of this outbreak is that the field had been maintained in the Conservation Reserve Program for the previous eleven years as a mixture of intermediate wheat grass and alfalfa. Perithecia formed abundantly on soybean residues in spring 2010, but fruiting was delayed in surface exposed residues that did not remain continuously moist. Epidemic levels of Northern stem canker occurred in no-till and conventional tillage plots established in 2007 on a scale of 0 to 10 during late summer/early fall. Data analysis indicated that there was significant yearly variation in the severity of CSNN and differences
Yearly variation in the development of current season needle necrosis on noble, Nordmann and Turkish fir Christmas trees in the U.S. Pacific Northwest
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Phytopathology 101:S31
Current season needle necrosis (CSNN) is a poorly understood disease that affects a number of Abies spp. that are grown as Christmas trees. CSNN has been reported on noble (A. procera), Nordmann (A. nordmanniana), and grand fir (A. grandis) in Europe and these species, plus Turkish fir (A. brutia) in Turkey. In the U.S. Pacific Northwest, in 2002 and 2004, a series of replicated genetic field trials were established at the Washington State University Research Center in Puyallup, WA. This is a low elevation site that is conducive to the development of CSNN. These trials contain 91 sources of noble fir, 15 sources of Nordmann fir and 4 sources of Turkish fir. CSNN data were collected annually for six years on the noble fir trees and five years on the Nordmann and Turkish fir trees. Disease severity was rated on a scale of 0 to 10 during late summer/early fall. Data analysis indicated that there was significant yearly variation in the severity of CSNN and differences

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in the susceptibility of the different sources of trees in these trials to CSNN. Spearman rank order correlation analysis indicated that there was a highly significant correlation between the yearly susceptibility rankings of the sources during the years data were collected on the noble, Nordmann, and Turkish firs. These results indicate that the relative susceptibility of different sources of trees to CSNN can be determined after one or two years at conducive sites.

**Spread of Phytophthora ramorum to water, soil, and vegetation outside a nursery in Pierce County, Washington**

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Phytopathology 101:S32

Since its initial detection in a nursery in 2003, Phytophthora ramorum has been found in 48 nurseries, four streams, and three drainage ditches in Washington. In 2004, P. ramorum-positive plants were confirmed at a Pierce County nursery. For four years the nursery was inspected and no additional P. ramorum was detected, however, during 2009 symptoms of P. ramorum were observed on plants that had been inspected in the nursery. In 2010, additional plants in the nursery were confirmed to be infected with P. ramorum. The positive salal along the ditch was removed during the spring of 2010. In 2011, all plants were positive on the nursery, and ditch water continued to be positive along the perimeter of the nursery. Composite soil samples collected from a small drainage ditch were positive in 2010; making this the first location in Washington where evidence that inoculum has spread from a nursery resulted in contamination of water and soil and infection of natural vegetation. The positive salal on one of the plots was sampled, and samples genotyped in 2010 from the perimeter of the nursery were of the NA1 lineage. The positive salal probably is the source of inoculum associated with the detection of this pathogen in a Washington State waterway.

**Mystery of the Sammamish: What are the sources of Phytophthora ramorum infesting this Washington State waterway?**

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Phytopathology 101:S32

Phytophthora ramorum was first detected in Washington in 2003 and has been detected since in 48 nurseries, four streams and three drainage ditches. Genotype analysis indicates that contamination of waterways has typically resulted from spread of inoculum from nearby positive nurseries. However, the source of inoculum associated with the detection of this pathogen in a four-mile-long section of the Sammamish River is a complex situation that remains unresolved. There have been at least nine P. ramorum-positive nursery sites in the Sammamish watershed since 2004. The initial detection of P. ramorum in the river in 2007 was an NA1 genotype that was consistent with the genotype detected in a holding pond four miles upstream that drains from a positive nursery into the Sammamish River. Baiting in 2008, 2009, and 2010 detected additional NA1 and two additional genotypes of the pathogen in the river. Positive baits at the mouths of two streams and a drainage ditch that runs through an industrial site into the river in 2009 and upstream in these waterways in 2010, indicate that there are multiple sources of inoculum that have contaminated the river. The NA2 in the river appears to be coming from one of the streams, which drains an area with a NA2 positive nursery approximately three miles upstream from the river. Efforts are underway to identify the sources of inoculum that have contaminated the other stream and the industrial ditch.

**LuxR homolog XagR of Xanthomonas axonopodis pv. glycines is solubilized only in the soybean plant and contributes to the infection process**

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Phytopathology 101:S32

Xanthomonas axonopodis pv. glycines (Xag) is the causal agent of bacterial pustule of soybean. To better understand the virulence factors of Xag in the soybean pathosystem a luxR homolog, termed xagR, encoding a putative transcriptional regulator was studied. Disruption of xagR in Xag strain 12-2 resulted in a significant reduction of incidence of infection of soybean. While the transcription of xagR appears constitutive, XagR accumulates and is solubilized only in soybean plants and could not be induced by plant extracts in culture. Apparently some component(s) in soybean plant are involved in stabilizing XagR from proteolytic degradation, thus increasing protein levels rather than enhancing its transcription. Both pip (proline iminopeptidase) and pro (extracellular protease) genes as well as biosurfactant production on swarming plates exhibited enhanced expression in xagR-over-expressing strains compared with the wild type. XagR over-expressing strains also incited significantly few lesions on soybean.

**Host plant and substrate mediated shifts in soil microbial community composition in microplots simulating transitional organic production systems**

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Phytopathology 101:S32

Increased pest damage during transition from conventional to organic systems is an impediment to the implementation of organic farming practices. A micro-plot experiment was conducted to determine if damage from soilsborne pests can be mitigated through crop production practices that impact soil microbial communities. Three rates of urban plant debris and broiler litter and two planting regimes (continuous tomato or a rotation of sunn hemp (Crotalaria juncea) and Japanese millet (Echinochloa crusgalli)) were established in microplots previously planted to tomato (Solanum lycopersicum) for 18 continuous months. Soil in plots was solarized annually. Fungal and bacterial DNA was extracted from soil at periodic intervals, amplified using LH-PCR, and subjected to fragment analysis. Changes in soil bacterial communities were evident following amendment of broiler litter (nitrogen-mediated) or the plant host (rhizosphere-mediated). Changes in soil fungal communities were associated with the addition of urban plant debris (carbon-mediated). After 28 months, all plots were planted to tomato and damage from soilsborne pests monitored. An interaction of plant host and urban plant debris significantly impacted bacterial wilt, caused by Ralstonia solanacearum. Disease incidence was higher in plots containing urban plant debris, except where sunn hemp/millet was previously planted, indicating a complex association with practices impacting soil microbial communities.

**Protein-protein interaction of Cucurbet aphid-borne yellows virus using yeast two-hybrid system and bimolecular fluorescence complementation**

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Phytopathology 101:S32

In this article, yeast two-hybrid system (YTHS) and bimolecular fluorescence complementation (BiFC) were used to analyze interactions of Cucurbet aphid-borne yellows virus (CBYV)-encoded proteins. P0, P1, P1-2, P3, P4, and P5 were tested by YTHS in all possible pairwise combinations, and interaction was detected only for the P3/P3 combination. Results obtained by BiFC further confirmed the P3 self-interaction, and the subcellular localization of recomnstructed YFP complexes was observed mainly in nuclei of Nicotiana benthamiana leaf epidermal cells. Domains involved in P3 self-interaction were analyzed using deletion mutants by YTHS and BiFC. The results showed that R domain (residues 1–61) in the N-termini interacting with itself and with S domain (residues 62–199) in P3, was responsible for P3 self-interaction. The present work would serve as a molecular basis for further characterization of CAYBV proteins, and the regions in P3 self-interaction could provide the clue for understanding the capsid assembly pathway of CAYBV

**Evidence that recombination plays an important role in the evolution and emergence of new curtoviruses (family Geminiviridae)**

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Phytopathology 101:S32

Curly top disease is caused by a complex of leafhopper-transmitted curtoviruses (family Geminiviridae). A putative new curtovirus (BV3) associated with curly top disease in tomato in California was identified in 2003. A four-mile-long section of NA2 indistinguishable from the reference isolate was infected, and induced typical curly top symptoms in Nicotiana benthamiana plants. The BV3 clone was 2931 nucleotides and had a typical curtovirus genome organization. This genome sequence comparisons revealed that it is most similar (~96%) to a putative new curtovirus species, Pepper curly top virus, previously identified from pepper in New Mexico. Interestingly, sequence comparisons of BV3 with other curtoviruses revealed high levels of identity (95–100%) with the C1 and C4 open reading frames, right inner gene of Beet severe curly top virus (BSCVT-[US:Ch]), revealing a recombination event between
these curtoviruses. Host range experiments involving agroinoculation and leafhopper transmission revealed that BV3 has a similar host range to BSCTV-[US:Cfh], including a severe symptom phenotype in sugar beet. A second recombinant curtovirus isolate was identified from beet leafhoppers collected from the Central Valley of California in 2010. The genome of this isolate was composed of Beet mild curly top virus (major parent) and BV3 (minor parent), and it may represent another new curtovirus species. Together, these results suggest a more important role for recombination in evolution and emergence of new curtoviruses than previously recognized.

Characterization of the occF gene associated with antifungal activity of occidiofungin produced by Burkholderia contaminans strain MS14
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Phytopathology 101:S33
Occidiofungin produced by Burkholderia contaminans strain MS14 is an octapeptide with a xylose and shows a broad range of antifungal activities to plant and animal fungal pathogens. The 56-kb occ gene cluster required for occidiofungin production harbors five nonribosomal peptide synthetase genes (occA-occE), two regulatory genes (ambR1 and ambR2), one cyclic peptide transporter gene (occT) and other unknown genes. The occF gene, 657 bp in size, is located downstream of occA. The putative protein encoded by occF shares 95.0% and 93.6% identities to glycosyl transferases of Burkholderia ambifaria strain AMD and B. abounensis strain Bu, respectively. It was hypothesized that occF adds xylose to the oligopeptide backbone of occidiofungin. To test the hypothesis, a nonpoler gptII cassette was inserted into the occF reading frame to generate the occF mutant MS14K1C1 (occF:gptII). Plate bioassays showed that antifungal activity of the mutant MS14K1C1 against the indicator fungus Geotrichum candidum was significantly reduced as compared with the wild-type strain MS14. Mass analysis using matrix-assisted laser desorption/ionization confirmed the lack of xylose in the occidiofungin produced by the mutant MS14K1C1. These data suggest occF is responsible for addition of xylose to occidiofungin and the presence of xylose is important for the antifungal activity of occidiofungin. This work provides insights for development of biofungicides.

Describe, classify and cultivation of Chinese and America Edible Mushroom 380 species of Inner-Mongolia, Yunan, Tibetan and California and Alaska
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Phytopathology 101:S33
The edible mushroom is the most attractive and popular health food. Those who work in high-tech fields of DNA-based studies do not understand the various names of the mushrooms. Therefore, this study, which requires listening, reading phonetically, and viewing a detailed dictionary, provides new information. Here, we provided the taxonomy of each edible mushroom with clarification of China’s ten-thousand-year-old agricultural history of cultivation, taming, and practical uses of edible mushrooms. This five year study includes each species’ name (Scientific, English and Chinese), nutritional and medicinal value, and requirements for cultivation techniques including 55 internationally marketed edible mushrooms species, and 245 wild, edible species and varieties. University of California (California 49 and Alaska 46) and China (Shenyang Japan 38 and Sino-Himalaya 59). Also included are classified indices for over 700 edible and medicinal species classified by the AFOTAL and shared with a total of 263 American species including 45 marketed edible mushrooms species, 43 medicinal, and 23 species that are both edible and medicinal as well. This study continues to aid and examine the ecology and molecular biology and phylogeny system (even fossils), and seeks to do more to stabilize nomenclature.

The study of Tibetan Plateau forest disease and insects and it’s integrated pest management
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Phytopathology 101:S33
The Tibetan (Qinghai-Xizang) Plateau, known as the “world’s roof,” contains a treasure for the natural sciences. The research was carried out in 2000–2006. The study on forest disease and insect was 231 km, with 124 forest sample spots areas. The study were done in 26 main virgen forest ecotypes, 62 plantation ecotypes, and 31 nurseries, fruits and tea gardens. More than 851 diseases and fungal specimens and 118 insect specimens were collected. Identification of them was made either in the field or at the Forest pathology Lab, (CAF) and Beijing Agricultural University, with involvement of many experts in forest pathology, mycology, and entomological laboratories. Tibetan are biodiversitys

Diffrential proteins and genes related to Curcularia lunata potential virulence variation induced continuously by resistant maize germplasm
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Phytopathology 101:S33
To understand the potential risk of genetic variation of Curcularia lunata in maize particularly as resistant maize varieties with the similar genetic background are widely and continuously planted, firstly we continuously carried on subinoculation with weak virulence strain (WS18) to 4-5 leaf age leaves of resistant inbred lines Pob21, Pob 43 and Pob 101, respectively, for eleven generations, then detected differential proteins and genes between re-isolated strain generations using proteomic and transcriptional level assays. Results suggested that as the growth of strain generation, the virulence was gradually enhanced, and up to peak at the six fourth generation. The proteomic analysis showed that among twenty eight differential proteins identified by MS/MS, eleven proteins were up-regulated, and fifteen down-regulated, and two unique, which Bn1, Bn2 and SCG as well as HSP 70, peroxiredoxin TSA1 and SOD were all speculated to be involved in pathogen virulence differentiation. SHI was constructed and fourteen differential genes were expressed, in which Bn1, ubiquitin, laccase-1 precursor, peroxiredoxin TSA1, SOD, etc., were up regulated association with pathogen virulence variation. We preliminarily selected some of them being identified through gene deletion and mRNA expression analysis, results revealed that Bn1, SCG and SOD were more closely involved in the pathogenic variation induced on resistant maize.

Construction and function analysis of Trichoderma transformant with Metarhizium anisopliae genes against insects
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Phytopathology 101:S33
Trichoderma is not always effective in practical application particularly when insect pests and diseases occur simultaneously at the same time and space. Aiming to the problem we conducted the transformation of chit42 or pr1 cloned from Metarhizium anisopliae into Trichoderma koningi and examined both genes expression in Trichoderma transformants. The average chitase activity of transformants is approximately 2 times over wild type strain, of which TMC42-4 and TMC42-11 exhibited highest chitase activity at 6-day of incubation in medium. Maize borer larvae were used to assess lethal effect of transformants to the pest. Among all transformants, TMC42-4 and TMC42-11 exhibited the most significant lethal effect to the larvae after two day -feeding. Electron microscope analysis of corn borer mid-gut after feeding by transgenic Trichoderma showed that goblet cells enlarged, microvilli of mid-gut began to fall off and mid-gut cell nucleus elongated. Meanwhile, the transformants with chit42 from Metarhizium anisopliae still maintained a significant antagonistic activity to Fusarium verticilloides and Fusarium graminearum, two major maize stem rot pathogens. For further improving transformant inhibition efficiency to maize borer, we linked five chitin-binding domains of different organisms to chit42, respectively, then transferred each of fused genes into Trichoderma wild type strain. Results suggest that transformants with chit42-Bm and chit42-Dm showed the highest chitinase activities.

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Phytopathology 101:S33
Wheat leaf rust, caused by Puccinia triticina, is an important foliar disease of wheat in China. The dynamics of races and virulences in the P. triticina populations in China during 2000-2006 were studied. Leaf rust samples were collected during surveys of wheat fields and trap nurseries in 17 provinces, and provided by coworkers throughout China. The virulence of single-pustule isolates was determined on near-isogenic Thatcher lines for leaf rust resistance genes Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, and Lr30, and races were denominated using the Prt code system. During 2000–2006, 79 races were identified from a total of 613 isolates. Races PHT (23.7%), THT (14.7%), PHI (11.4%) and TJI (4.2%) were the four common races, all avirulent to Lr9 and Lr24. The frequency of isolates with virulence
to Lr1, Lr2c, Lr3, Lr11, Lr16, Lr17 and Lr26 was over 80% and these isolates were widely distributed in China, while the frequencies of virulence to Lr9, Lr19, Lr24, Lr25, Lr28, and Lr29 were 0.2 to 2.5%. The diversity of virulence phenotypes of Chinese *P. triticina* populations appeared to increase from 2000 to 2006. Regional distribution patterns of races and virulences within China and between China and other countries suggest that the populations of *P. triticina* in China are isolated from these countries.

**Baseline sensitivity and potential resistance mechanism of *Monilinia fructicola* to SYP-Z048**

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Phytopathology 101:S34

SYP-Z048, chemical name 5-(4-chlorophenyl)-2,3-dimethyl-3-(pyrindine-3)-oxazoline, is a novel oxazole derivative fungicide inhibiting ergosterol biosynthesis in fungi. In this study, the baseline sensitivity of 100 *Monilinia fructicola* isolates from China to SYP-Z048 was established by measuring mycelial growth inhibition on Yeast Glucose medium. Laboratory mutants resistant to SYP-Z048 were generated and characterized. The sensitivity of mycelium ranged from 0.0034 to 0.0470 µg/ml with a mean EC50 value of 0.0188 µg/ml. Mutants resistant to SYP-Z048 were generated using UV-irradiation, but no mutants were obtained spontaneously. All fitness parameters evaluated in this study pointed to reduced fitness in mutants. Resistant mutants with EC50 values greater than 0.3 µg/ml exhibited a single point mutation in the ERG11 gene encoding the sterol 14α-demethylase resulting in an amino acid (aa) change from tyrosine to phenylalanine at position 136 (Y136F). No other mutation associated with resistance was detected in the CESA1, CESA2 and CESA4 genes. The mutations were verified by genetic cross assay. One parent (838) is a resistant homozygous strain at position 1105 and 1109, and the other parent (22) is a sensitive homozygote. All of F1-progenies from the cross [838(R) × 22(S)] showed resistance to SYP-Z048 and propiconazole (R2 = 0.87). In this study, the baseline sensitivity of 100 *Monilinia fructicola* isolates from China to SYP-Z048 was established by measuring mycelial growth. The sensitivity ranged from 0.0034 to 0.0470 µg/ml with a mean EC50 value of 0.0188 µg/ml. Mutant resistant to SYP-Z048 were generated using UV-irradiation, but no mutants were obtained spontaneously. All fitness parameters evaluated in this study pointed to reduced fitness in mutants. Resistant mutants with EC50 values greater than 0.3 µg/ml exhibited a single point mutation in the ERG11 gene encoding the sterol 14α-demethylase resulting in an amino acid (aa) change from tyrosine to phenylalanine at position 136 (Y136F). No other mutation associated with resistance was detected in the CESA1, CESA2 and CESA4 genes. The mutations were verified by genetic cross assay. One parent (838) is a resistant homozygous strain at position 1105 and 1109, and the other parent (22) is a sensitive homozygote. All of F1-progenies from the cross [838(R) × 22(S)] showed resistance to SYP-Z048 and propiconazole (R2 = 0.87). No other mutation associated with resistance was detected in the putative resistance genes of ERG2, ERG24, and ERG27 encoding A8 → A7 sterol isomerase, A14-sterol demethylase, C-14 sterol reductase, or 3-keto reductase. Cross resistance was verified between SYP-Z048 and propiconazole (R2 = 0.87). Cross resistance was verified between SYP-Z048 and tridemorph, carbendazim, procymidine, azoxystrobin, or pyrimethanil. The lack of ability of *M. fructicola* to generate spontaneous SYP-resistant mutants coupled with reduced fitness of F136Y mutants offers an explanation for the lack of such mutations in field populations. Our results show that SYP-Z048 is a DMI fungicide with potential for brown rot control in stone fruits.

**Evaluating alfalfa cutting as a potential measure to enhance abundance of predators to *Aphis gossypii* in cotton-alalfa intercropping system**

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Phytopathology 101:S34

The effects of alfalfa-cutting and non alfalfa-cutting on the population dynamics of *Aphis gossypii* Glover (Homoptera: Aphididae) and several species of arthropod predators were examined in cotton-alalfa intercropping pattern in north-western China. The objectives of this study were to evaluate the alfalfa-cutting as a potential management technique to enhance abundance of arthropod predators of *A. gossypii*. The entire study area consisted of 50-cm interspace between alfalfa cut strips and cotton. Alfalfa cut strips were cut in June and July, when the population density of *A. gossypii* was gradually increasing in cotton, forced some groups of predator to migrate into adjacent cotton fields from alfalfa. Individual number, species richness, and diversity of predators were higher in alfalfa cut cotton field than in uncut. The population density of *Adonia variegata* (Goeze) (Coleoptera: Coccinellidae), *Parlatoria indica* (Aurantze: Lycosidae), *Chrysopus sinicus* Tjeder (Neuroptera: Chrysopidae) and *Orias minutus* (L.) (Hemiptera: Anthocoridae) increased 120%, 101%, 61% and 7% in alfalfa-cutting than non alfalfa-cutting respectively. Meanwhile, the population density of *A. gossypii* in non alfalfa-cutting was 2.8 times larger than in alfalfa-cutting. This indicates that alfalfa-cutting induces predator immigration into adjacent cotton fields and helps control cotton aphids. This study provides cotton growers a potential cultural management technique for *A. gossypii* while conserving predators.

**Creeping stem cuttings, the possible inoculum source for bacterial wilt of *Ipomoea batatas***

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Phytopathology 101:S34

Searching for small RNAs in *Xylella fastidiosa* genomes

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Phytopathology 101:S34
Pierce’s disease of grapevine. However, little is known about small RNA in this bacterium, despite the fact that several whole genome sequences of X. fastidiosa strains have been published. To fill in this gap, a research project was initiated to search for small RNAs in Xylella fastidiosa. The complete genome sequences of four X. fastidiosa strains (9asC, M12, M23, and Temecula1) were selected for in silico analysis to scan for small RNA using the sRNAscan program (PLOS ONE 5:e1970). Candidate small RNA genes were identified in all of the four X. fastidiosa strains with 46 for 9asC, 50 for M12, 49 for M23, and 47 for Temecula1. Size of candidate small RNA ranged from 40 to 350 bp. Results from BLAST analysis showed that 34 small RNA genes were shared by all four X. fastidiosa strains. Species-, subspecies- and pathotype-specific small RNAs were also identified.

Genetic structure of Waitea circinata var. circinata on creeping bentgrass and annual bluegrass putting greens in southern California

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Phytopathology 101:S35

Brown ring patch is a new disease of annual bluegrass, rough bluegrass and creeping bentgrass in the U.S. caused by Waitea circinata var. circinata (Wcc). An initial study found significant haplotype diversity (n = 17) and a lack of population structure from 42 Wcc isolates broadly sampled across the U. S. This is not consistent for a newly introduced pathogen. In this study, soil samples were collected from two putting greens at a course in La Jolla, CA for 3 years to obtain distinct pathogen populations. In total, 116 Wcc isolates were recovered from the 465 soil cores. Amplified fragment length polymorphism was used to analyze the genetic structure. Based on 53 loci, most isolates were found to be unique genotypes. After clone-correcting the data set and analyzing 10 loci with allele frequencies between 0.29 and 0.86, no significant pair-wise population differentiation was found based on Fst analyses (0.08 < theta < 0.06), consistent with gene flow occurring between greens and between years. In contrast, multilocus disequilibrium analyses based on the index of association were only consistent with a random mating model from 3 out of the 6 populations. However, high genotypic diversity and few clonal genotypes found between years suggest that the pathogen may be sexually reproducing in the field and that inbreeding could account for the gametic disequilibrium detected.

Development of expressed sequence tag-tagged SSR markers for Puccinia striformis, the stripe rust pathogen

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Phytopathology 101:S35

Puccinia striformis causes stripe rust on wheat, barley, and many grass species. Co-dominant microsatellite markers are more suitable for studying population structures of this dikaryotic fungus. In this study, we characterized simple sequence repeat (SSR) loci based on three previously developed expressed sequence tag (EST) libraries for P. striformis f. sp. tritici (Pst), the wheat stripe rust pathogen. SSR loci were isolated by screening the EST sequences using the Simple Sequence Repeat Identification Tool. By scanning 3,311 unique EST sequences, filtering out those with repeat sequences less than 18 bp, and screening those with suitable flanking sequences, 46 were selected for designing primers using the Primer3 program. The 46 primer pairs were tested and 44 were successful for Pst and 60% of the 46 were successful for P. striformis f. sp. hordei using the M13 tailing and fluorescent capillary electrophoresis on an ABI3730 genetyper. The three PST races represent the most diverse races of the wheat stripe rust pathogen from 1960s to 2007 in the U.S. Thirty-four primer pairs produced repeatable polymorphic bands and 19 of them generated co-dominant markers among the four races. The lengths of the amplicons ranged from 130 to 506 bp containing 5 to 13 di-, tri-, or tetra-nucleotide repeat SSR. primers should be useful in studying the population structure and evolution of the pathogen.

Aspects of popcorn disease occurrence on mulberry fruits in Korea

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Phytopathology 101:S35

Occurrence of popcorn disease on mulberry fruits was surveyed at Jeollabukdo location in Korea from 2009 to 2010. The diseased flowers turned grayish white and changed to hard and black sceloria during overwintering after falling onto the ground. A popcorn disease was occurred up to 90 percent from late May to late June. Incidence was 20–90 percent when a mulberry tree was cultivated in the raising outdoors but was occurred weakly in the vinyl plastic hothouse. Incidence of popcorn disease according to the height of mulberry tree was high three times near by the ground (0~70 cm) than that of 140 cm upper side on the three height. Apothecia produced from overwintered sceloria in the fields of mulberry trees were observed in late April. Two types of apothecia were produced from the sclerotia, which were cup-shaped or clump-shaped. The fungus with cup-shaped apothecia was identified as Ciboria shiraiana, and that with clump-shaped apothecia as Sclerotinia shiraiana. C. shiraiana and S. shiraiana occurred at the ratio of about 7 vs. 3 in the fields.

Deletion of the N terminus of Papaya ringspot virus larger coat protein disrupt viral systemic infection

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Phytopathology 101:S35

The NlA protease of Papaya ringspot virus (PRSV) is responsible for the processing of at least six cleavage sites in the C-terminal part of the polyprotein. According to the cleavage rule of NlA, two conserved cleavage sequences (VYH/E and VFHQ/S) exist in the region between NlB protein and coat protein (CP) and resulted in the formation of two in-frame heterologous N-terminal CP with 20-amino-acid apart. The purpose of our study is to characterize the role of the two CP in PRSV during virus infection. Five CP mutated viruses were constructed, including CPΔ10-10, CPΔ10-10, CPΔ10, CPΔ10-10 and CPΔ10. Plasmids contained wild type genome (CPΔ10-10) or five CP mutants were inoculated into systemic host Carica papaya for examining the symptom expression and virus accumulation. Plants inoculated by CPΔ10-10 showed typical symptoms at 10 d.p.i., while plant inoculated by CPΔ10-10, CPΔ10, CPΔ10-10 and CPΔ10 resulted in only 10% or the other mutants were unable to cause any symptoms. Western blot and ELISA analyses using an antiserum against PRSV showed that all the mutant viruses, except for CPΔ10-10, were existed in the inoculated leaves, while only wild type, CPΔ10-10 and CPΔ10-10 mutants were detectable in the systemic leaves. Our preliminary results suggested that the mutations in the N terminus of larger CP did hamper the ability of PRSV infection in plants and the 20 amino acids at the N terminus of the larger CP play a critical role in viral systemic infection.

Diversity of the mating type locus in Sclerotinia sclerotiorum in relation to formation of apothecia

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Sclerotinia sclerotiorum is a homothallic fungus and carries both alpha box (MAT1-1) and HMG box (MAT1-2) genes together at a single MAT locus. We investigated the presence of these genes for S. sclerotiorum isolates from lettuce. In addition, we also investigated the MAT locus diversity among the eight sibling ascospores harvested from a single ascus. Primer sets from the literature that previously resulted in amplification of MAT1-1 and MAT1-2 genes in S. sclerotiorum were employed in this study. We found that MAT1-1 positive isolates failed in approximately 25 to 50% of the isolates depending on the sampled field. Likewise, among the eight sibling ascospores, only 4 ascospores showed the MAT1-1 amplicon, suggestive of segregation. In contrast, the MAT1-2 specific primers produced amplicons for all isolates, including all eight sibling ascospores. There was a correlation between the lack of MAT1-1 amplification and the formation of apothecia in culture. Furthermore, nearly all MAT1-1-positive isolates formed apothecia, only 28% of the isolates that were MAT1-1-negative produced apothecia. All eight sibling ascospores produced apothecia. We are currently using DNA sequencing to investigate the MAT locus variability in relation to apothecia formation.

Analysis of gene expression during infection of field pea roots by Fusarium graminearum

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Field pea (Pisum sativum), an important rotational crop with cereals is greatly affected by root rots. Fusarium graminearum, commonly known as a cereal pathogen, has recently been associated with this disease. A study was conducted to elucidate mechanisms associated with F. graminearum infection on this crop. F. graminearum genes expressed exclusively during infection of field pea roots were identified and compared to genes expressed during infection of cereals. Fungal gene expression in artificially infected field pea roots and F. graminearum grown in culture was assessed using the Illumina RNA-Seq technology. A total of 3237 F. graminearum genes were found to be differentially expressed in planta. Among these, 1627 genes were upregulated and 1610 were downregulated. The upregulated genes included homologs of genes encoding virulence factors in Nectria haematococca such as, an ABC transporter homologous to the MrbCI gene and several pinastin demethylase homologs similar to the PDA1 gene. Other upregulated genes
with known functions included those involved in cell wall degradation like endoglucanases, xylanases, and pectate lyases. Expression of six upregulated genes was confirmed by RT-PCR to validate the inferences from the sequencing results. Comparison of genes expressed during this interaction with those common between crown rot of wheat and head blight of barley suggests significant differences in expression patterns on this host.

Specific detection of the causal agent of bacterial blight, *Pseudomonas syringae* pv. *pisi* in the seeds of peas by nested PCR and real-time TaqMan PCR

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*Pseudomonas syringae* pv. *pisi*, a causal agent of bacterial blight of common peas, cowpea, and other leguminous cultures, is disseminated by the infected seeds. In this study, we have developed nested PCR and real-time TaqMan PCR for detection of the bacterium from the pea seeds. Primers pisi-JH-F and pisi-JH-R amplified a 306 bp fragment only from *Pseudomonas syringae* pv. *pisi* strains, while no amplification was occurred from the other plant bacterial pathogens. Nested-PCR with primers pisi-JH-F-ne and pisi-JH-R-ne amplified a 104 bp fragment only from the *Pseudomonas syringae* pv. *pisi* strains with 1000 fold more sensitive than 1st PCR. The primers did not amplified any non-specific DNA from the seed extracts of 8 different beans and peas and DNA isolated from the seed extracts. Real-time PCR using TaqMan probe-pist, which was designed inside amplicon of the nested PCR, amplified only from *Pseudomonas syringae* pv. *pisi* strain. Detection limit of the real-time TaqMan PCR was similar to the nested PCR’s with the artificially inoculated pea seeds. We believe that the PCR assays developed in this study are very useful to detect *Pseudomonas syringae* pv. *pisi* from the leguminous seeds.

Use of silver nanoparticles for control of seedborne diseases

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Optimal control of bacterial or fungal seedborne pathogens can be achieved by seed treatment to disfet the seed at the stage, because they are impossible or difficult to control after infection. Conventional seed treatment methods using fungicide, hot water or bleach have limited applications due to narrow control spectrum of target pathogens, harmful chemical exposure or contamination. Silver has highly effective antimicrobial properties but low toxicity to humans and plants. If silver is transformed into a nanoparticle, its antimicrobial activity is intensified, making it useful in eliminating seedborne pathogens. The objective of this study was to evaluate antimicrobial activities of silver nanoparticles to control fungal (*Fusarium moniliforme*) and bacterial (*Burkholderia glumae*) pathogens in rice and their potential phytotoxicity to affect seed germination and seedling growth. Silver nanoparticles were chemically synthesized and their antifungal and antibacterial properties tested. Silver nanoparticles significantly reduced the fungal and bacterial pathogens both on growth media and rice seeds at concentrations of 0.15 – 150 ppm. Seed health after treatment was evaluated by germination rate, root and shoot lengths at 7 d after germination and seedling height at 3 wk after germination. No adverse silver effect on seeds was observed. These data suggest that silver nanoparticles can be used for seed treatment for control of various seedborne pathogens.

The transcription factor Amr1 induces melanin biosynthesis and conidium production but differentially suppresses virulence in *Alternaria brassicicola*

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*Alternaria brassicicola* is a successful saprophyte and necrotrophic pathogen with a broad host range. Some consider that, like many opportunistic plant pathogens, *A. brassicicola* is in a continual process of adaptation as a parasite. To better understand pathogenesis mechanisms in *A. brassicicola*, we screened targeted gene knockout mutants corresponding to over 200 predicted transcription factors. We discovered mutants of a gene *Amr1* with an unexpected increase in virulence that produced consistently larger lesions than the wild type. Lesions produced by the wild type were of various sizes, whereas lesions caused by the mutants were less variable than the wild type. The *amr1* mutants were melanin-deficient in all tissues and structural genes associated with the melanin biosynthesis pathway were not induced. In contrast, *amr1* mutants expressed higher amounts of several hydrolytic enzyme-coding genes in planta and grew faster than the wild type in the presence of pectin in an axenic medium. This study demonstrates that a gene important for survival in nature negatively regulates virulence by suppressing the expression of genes including a subset of those corresponding to cell wall-degrading enzymes. We speculate that the functions of this gene are important for *A. brassicicola* to be a successful saprophyte and opportunistic plant parasite.

The land plant-specific NbPSL1IP protein plays a key role in plant antiviral defense by interacting with *Potato virus X* RNAs and proteins

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Phytopathology 101:S36

*Potato virus X* (PVX) contains cis-acting elements including stem-loop 1 (SL1) RNAs at the 5′ region that are required for PVX RNA replication, encapsidation. A. to embracing A - a two-dimensional electrophoresis Northwestern blot analysis was used to identify 24 tobacco host proteins that interact with SL1 RNAs. The functions of one of these, NbPSL1IP, which binds to both SL1(+) and SL1(-) RNAs, was further characterized. A Blast search identified 24 land plant-specific NbPSL1IP homologous proteins. Phylogenetic analysis demonstrated that duplication of PSL1IPs has occurred less often in eudicots than in monocots or bryophyta. qRT-PCR revealed high induction of NbPSL1IP expression upon PVX infection. PVX RNA accumulation and movement were reduced by overexpression of NbPSL1IP and increased by silencing of NbPSL1IP, indicating a function of NbPSL1IP in antiviral defense. Subcellular localization studies demonstrated that NbPSL1IP is associated with microtubules and plasmodesmata. In addition, PVX infection altered the subcellular localization of NbPSL1IP from cytoplasmic to endoplasmic reticulum. The BIFC assay showed that NbPSL1IP interacts with multiple PVX proteins including capsid protein, TGB1, and TGB2. The land plant-specific NbPSL1IP inhibits PVX RNA accumulation and movement by interacting with cis-acting elements and PVX movement proteins and by re-localizing in response to PVX infection.

Diversity and pathogenicity of *Fusarium* species associated with grain mold of sorghum in Korea

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Phytopathology 101:S36

Sorghum (*Sorghum bicolor* Moench) is cultivating at present throughout Korea as a food and feed crop, which was traditionally grown on a small scale. Grain mold symptoms of the plant were frequently observed during disease surveys in Korea from 2007 to 2009. The symptoms were highly variable. Severely infected grain is fully covered with mold and partially inflected grain may look normal and discolored. Fifty-five isolates of *Fusarium* species were obtained from the disease symptoms of the plant collected from several locations in the country. Out of the isolates, 25 were identified as *Fusarium thapsinum*, 14 as *F. proliferatum*, 8 as *F. graminearum*, 5 as *F. equiseti*, and 3 as *F. incarnatum* based on their morphological and cultural characteristics. Elongation factor 1 alpha gene sequences of the isolates were used for phylogenetic analysis. Analyses of the sequences revealed that the isolates were confirmed to be identical with related species of NCBI GenBank. Pathogenicity tests showed that three competent species, *F. thapsinum*, *F. proliferatum* and *F. graminearum* were strongly virulent to grains of sorghum. The present study is the first report of sorghum grain mold caused by *Fusarium* species in Korea.

Detection of *Citrus leprosis virus* cytoplasmic type utilizing the polyclonal antibodies specific to the movement and coat proteins

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*Citrus leprosis virus* cytoplasmic type (CILV-C) is an economically important cytoplasmic virus in citrus growing areas in South and Central America. CILV-C is considered as one of the major exotic pathogens for introduction into the citrus industry. Detection is usually done by symptoms and reverse transcription polymerase chain reaction (RT-PCR). A standard serological diagnostic method is needed for the detection of CILV-C. Polyclonal antibodies were developed against expressed proteins of the putative coat protein gene p29 from segment RNA1 and the putative movement protein p32 from segment RNA2. To achieve this, the p29 and p32 gene sequences of CILV-C were synthesized, cloned into pDEST-17 vector and BL21-AI competent cells were transformed. The p29 and p32 expressed proteins were purified using His tag affinity chromatography after induction with L-
Improving PCR-based detection of Xylella fastidiosa in blueberry with a cost-effective DNA extraction procedure

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Phytopathology 101:S37

Commercial nucleic acid extraction/isolation kits are used widely because they are rapid (~30 min) and simple. However, they may not be suitable for plant tissues rich in polyphenols and/or polysaccharides, which are well-known PCR inhibitors. The high concentration of such inhibitors in blueberry tissue and xylem sap prompted us to evaluate various methods of DNA extraction to improve detection of Xylella fastidiosa (Xf), the causal agent of bacterial leaf scorch, an emerging disease. From healthy plants, leaf petioles (100 mg) were sampled and Xf suspensions added to final concentrations of 0, 0.6, 6, 60, 600, and 60,000 cells per qPCR reaction. In addition, petioles of symptomatic leaves from diseased plants were also sampled. DNA templates (100 µl) were obtained via three different plant kits (GenElute, Sigma-Aldrich; PowerPlant, Mo Bio; and DNasy, Qiagen), two soil kits (PowerSoil and PowerBiofilm, Mo Bio), Terra PCR Direct (Clontech), FTA cards (Whatman), and a modified CTAB protocol (adapted from doi:10.1016/j.viromet.2008.09.008). Using the CTAB procedure, all Xf samples were detectable with qPCR. Extracts from Terra PCR Direct and on FDA cards failed to amplify even from severely diseased samples. The other commercial kits were suitable for PCR-based detection greater than 600 Xf cells per reaction. Although relatively time-consuming (~1.5 h extraction), the sensitivity of the CTAB procedure permitted detection of Xf at 0.6 cells per reaction at low cost.

Double fliD and xagP mutants of Xanthomonas axonopodis pv. glycines and their roles on host and nonhost plant

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Phytopathology 101:S37

We carried out a genetic and functional characterization of the pectase lyase and hok associated protein 2 encoded by XagP and fliD of Xanthomonas axonopodis pv. glycines (Xag) strain 12-2 respectively. XagP has been reported to play a role in activating HR induction, where fliD functions as a capping structure at the distal end of the filament essential for motility. The inactivation of fliD led to reduced disease-associated pustule lesions on susceptible soybean cv. Spencer and still exhibited HR in tobacco leaves. The absence of xagP, expression of HR-like cell death was induced in cv. Spencer, but not in tobacco. Double mutation of these two genes also resulted in HR-like cell death in combination with no expression HR in tobacco leaves. Complementation to the corresponding deficient mutants restored Xag motility, pectolytic activity, HR, and virulence. This suggests a link between xagP and fliD in full virulence on soybean but not in HR on either host or nonhost plants.

A new endophytic fungus from Citrus medica var. sarcodactylis and its application on controlling damping-off and anthracnose of Brassica rapa

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Phytopathology 101:S37

The endophytic fungi are new sources of bioagent in management of plant diseases. A fungal isolate CB10 from the endophytic fungi is a new source of bi-agent in management of plant diseases. A fungal isolate CB10 from Citrus medica var. sarcodactylis (C. medica var. sarcodactylis) was identified as Appoharknessia spp. based on morphological and molecular characteristics. This fungus showed high efficiency in the inhibition of mycelial growth of several plant pathogens including Botrytis cinerea, Colletotrichum higginisianum, Fusic无辜 oxysporum and Pestalotiopsis psidii. A preliminary test on the control of anthracnose of Brassica rapa caused by C. higginisianum in greenhouse indicated that the disease incidence of B. rapa cv. San Fong No.2 was reduced by 37.5% four days after treatment with 100 µg/ml extract in comparison with untreated control (ME). Moreover, CB10 ME increased the survival rates by 38.9% of seeds (cv. San Fong No.2) in Rhizoctonia solani infected soil after seeds were soaked in 10 µg/ml ME. The bioactive compounds were identified by liquid chromatography/mass spectrometry/mass spectrometry/liquid chromatography after purification by column chromatography. The results showed five major compounds identified as m/z 195.1, 313.4, 314.4, 579.6 and 607.6. Based on data base of Spectral Database for Organic Compounds (SDBS), the five major compounds were identified as 2-hydroxy-5-thiocyanoatobenzoic acid, methyl trans-3-cyano-2-styryl-1-azulene carboxylate, methyl N-(4-(3-butyloxylo) sulfonyl)-alpha-methylbenzyl acetamide, azocarmine G, and disomine. The disomine was previously reported as one of flavonoid compound inhibit pathogenic mycelial growth and induce plant resistance.

Methods for introduction of nonpathogenic Fusicocus oxysporum into cucumber plants for better control of Fusarium wilt disease in Taiwan

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Phytopathology 101:S37

The nonpathogenic Fusicocus oxysporum (Fo) has been demonstrated to delay the symptom expression of Fusarium wilt of cucumber (Fusicocus oxysporum f. sp. cucumerinum) in greenhouse or field trials. For improving the inoculation system, three methods, by seed coating, substrate infestation or hypocotyl cutting, were compared for the efficacy of controlling Fusarium wilt of cucumber. The nonpathogenic isolate Fo276 that showed high potential in controlling the disease was used as an inoculum source and its distribution in inoculated cucumber tissues were analyzed. Results indicated that the hypocotyl cutting inoculation could reduce the severity of Fusarium wilt of cucumber by 64% whereas seed coating and substrate infestation only decreased the severity of Fusarium wilt by 20 and 41%, respectively. It was obvious that Fo276 provided better control of the disease when introduced by hypocotyl cuttings. Results of the distribution study indicated that Fo276 was restricted in peg tissue and mainly colonized in epidermis or cortex cell by substrate infestation and seed coating whereas Fo276 could colonize in hypocotyl tissue and vesicular tissues by hypocotyl cutting inoculation. Thus, the highly effective nonpathogenic Fo in hypocotyl and vesicular tissues via hypocotyl cutting may play a key role in a higher reduction of the disease severity.

Optimization of Maize fine streak virus (MFSV) protein expression in Drosophila S2 cells

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Phytopathology 101:S37

MFSV is a negative-sense RNA virus in the family Rhabdoviridae that is transmitted by the leaffopper G. nigrifrons. The virus replicates in both its maize host and insect vector. In order to determine whether Drosophila S2 cells can be used as a system to study MFSV replication, we tested conditions for optimal expression of MFSV proteins, a replicon construct and the T7 RNA polymerase. Two of the three proteins required for synthesis of the MFSV genomic RNA, MFSV N and P, could be expressed from pMT/V5-His-Topo vector in S2 cells for at least 4 days after induction with CuSO4. Experiments are underway to assess MFSV L protein expression in S2 cells. Preliminary results suggest that the L protein can be detected only when expressed from linear DNA fragments. The replicon construct carrying the MFSV vector transcriptional site expression HR resulted in a 28-fold increase in antisense GFP cDNA sequence downstream of a T7 promoter was inserted into the same vector. The transcript accumulated in transfected cells for at least 4 days. As expected, the GFP protein did not accumulate in transfected cells in the absence of the MFSV components and T7 polymerase. Expression of constructs encoding the T7 polymerase indicated that the protein could be detected in S2 cells at 24 h when fused to a nuclear localization signal peptide (NLS), but did not accumulate when lacking the NLS. Our results indicate that the proteins and constructs required for MFSV replication can be expressed in S2 cells.

Review of the development of fludioxonil for post harvest decay control on various tropical fruit crops

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Phytopathology 101:S37

Post harvest decay of tropical fruit including mango, papaya, and pineapple represents one of the most important challenges facing growers worldwide, particularly for transportation of these crops to distant markets. Fludioxonil (branded as Scholaf® and Graduate®, Syngenta) has been widely tested and particularly for transportation of these crops to distant markets. Fludioxonil being a resistance management tool, enabling rotation of this new mode of action. fludioxonil offers both long lasting control of postharvest decay, as well as being a resistance management tool, enabling rotation of this new mode of action. Volume 101, No. 6 (Supplement), 2011 S37
action with other registered products. This paper reviews studies conducted over the past eight years on various tropical fruits.

Seed storage duration and relationships with seed quality
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Two cultivars differing in seed quality were placed in four storage environments varying from ideal to poor. Seed were sampled every two weeks and tested for standard germination, accelerated aging, and with the Seed Vigor Imaging System (SVIS). Seed were also planted in the field and emergence determined at 2 and 4 weeks. Standard germination differed between the two cultivars, but did not change significantly throughout the test. Accelerated aging and SVIS remained relatively constant until the middle of July and then began to decline. The change in these parameters was greater for the poorer quality seed lot and was especially severe under the poorest storage conditions. Stands were generally higher in first two months with the higher quality seed lot, but stands of all seed lots declined sharply by the middle of July no matter how they were stored. Correlations of the seed quality assays and field performance were inconsistent in the SVIS test when data were analyzed by planting for the first emergence count. However, accelerated aging data did have a significant positive correlation with field emergence for every planting at the time of the first stand count. No correlations of the quality tests and field emergence for the data collected at the second stand count were consistently significant throughout the plantings.

Sexual reproduction of Pseudoperonospora cubensis
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The oomycete Pseudoperonospora cubensis attacks members of the Cucurbitaceae, especially cucumber and melon in which it causes severe foliage damage. It propagates clonally by sporangia. Sexual reproduction was not reported nor the germination of, or infection with, oospores. Here we report on the sexual reproduction of P. cubensis under controlled conditions in the laboratory. When field isolates were inoculated singly onto detached leaves in growth chambers at 20°C, sporangia were produced but not oospores. However, when pairs of selected isolates were mixed and inoculated onto detached leaves, oospores were formed in the mesophyll within 6–11 days. Oospores were round, light-brown, 40 μm in diameter. Oospores were produced in Cucumis sativum and Cucumis melo but not in Cucurbita pepo, Cucurbita moschata or Cucurbita maxima. Oospores-containing leaves were homogenized in water, the homogenate was dried twice to kill sporangia, re-

The role of lipopolysaccharide in virulence and host specificity of Xylella fastidiosa
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(1) University of California, Riverside, CA, U.S.A. Phytopathology 101:S38

Xylella fastidiosa (Xf) is a Gram-negative, xylem-limited bacterium that causes disease in a wide range of hosts, including grape, almond, and oleander. Both almond and grape isolates can cause disease in grapevines. However, grape isolates cannot cause disease in almonds, indicating a fundamental difference between these two isolates. Moreover, the oleander strain cannot infect grape or almond and vice versa. The molecular mechanisms that determine this host specificity are poorly understood. We are investigating the role of the abundant cell surface polysaccharide, lipopolysaccharide (LPS) in the interaction between Xf and its grape host. Because LPS is displayed on the cell surface, it mediates interactions between the bacterial cell and its surrounding environment. LPS is comprised of conserved lipid A-core component and a variable O-antigen portion. O-antigen has been implicated in virulence and host specificity in many bacterial species. By targeting key genes involved in O-antigen biosynthesis, we will determine if O-antigen is an important virulence factor for disease development in grape. More specifically, we are investigating the contribution of O-antigen to surface attachment and mature biofilm formation, two critical steps for successful infection of the host xylem. Additionally, we will determine if O-antigen contributes to the high level of host specificity observed for this pathogen.

Global food security short courses to enhance urban forestry education and training at Southern University and A & M College
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(1) Urban Forestry Program Southern University and A & M College, Baton Rouge, LA, U.S.A. Phytopathology 101:S38

There is a need to provide more post baccalaureate training and experience in plant health management at U.S. land grant universities to counter the threat of exotic or naturally occurring plant pathogens to ensure our national security and global competitiveness of U.S. Agriculture. The objective of the global food security short courses are to enhance urban forestry education and training in global food security by providing students with firsthand experience in a multidisciplinary, setting with various universities, state and local government agencies, and non-governmental organizations and by isolation. In one substrate sample, six were found to be positive for P. ramorum by both real-time and nested PCR and by isolation. In one substrate sample, P. ramorum was detected by nested PCR and not by real-time PCR or isolation. Overall, there was agreement among detection assays for 22/23 substrate samples (96%). Real-time and nested PCR appear to be as reliable as isolation on selective medium for detecting P. ramorum from leaf pieces used as soil baits. In addition, these two detection assays greatly reduce the time required to obtain results.

Phytophthora ramorum has been shown to be present in field soil and container mixes (i.e., substrates) at ornamental plant nurseries. P. ramorum can be recovered from a substrate using a baiting bioassay, which involves culturing of infected bait pieces on selective medium (PARP-V8). Results may not be available for 2–4 weeks. Techniques for the rapid detection of P. ramorum in substrates are needed to give regulators a better tool to prevent the spread of this quarantine pathogen. Two or three replicate aliquots from each of 23 substrate samples were baited with leaf disks from Camellia japonica and Rhododendron catawbiense. Some of the bait pieces from each aliquot then were embedded in PARPH-V8 to isolate P. ramorum, and DNA was extracted from other bait pieces for molecular detection of the pathogen. DNA was examined by both real-time and nested PCR using the ITS gene target following the USDA-APHIS protocol. Of the 23 substrate samples, six were found to be positive for P. ramorum by both real-time and nested PCR and by isolation. In one substrate sample, P. ramorum was detected by nested PCR and not by real-time PCR or isolation. Overall, there was agreement among detection assays for 22/23 substrate samples (96%). Real-time and nested PCR appear to be as reliable as isolation on selective medium for detecting P. ramorum from leaf pieces used as soil baits. In addition, these two detection assays greatly reduce the time required to obtain results.

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J. COLBURN-CLIFFORD (1), M. C. Roper (1)
(1) University of California, Riverside, CA, U.S.A. Phytopathology 101:S38

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Rhizoctonia web blight development on azalea in relation to duration of leaf wetness

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Phytopathology 101:S39

Rhizoctonia web blight, caused by binucleate *Rhizoctonia* spp., is an annual problem in the southern United States on container-grown azaleas (*Rhododendron* spp.). Temperature has been the primary variable for predicting web blight development in the field, with only minor predictive contribution due to moisture variables. To better define the influence of moisture on web blight development, plants were maintained in a greenhouse in open-topped, clear plastic chambers with 0, 4, 8, 12, and 20-h cycles of 10 sec. mist at 30-min intervals, all terminating at 9:30 A.M. Binucleate *Rhizoctonia AG*-U infested barley was secured in netting at the base of terminal leaf clusters, and a proportional leaf blight incidence per stem recorded as a repeated measure over 6 weeks. Temperature, relative humidity (RH), and leaf wetness (LW) were recorded in each chamber. A similar day (29°C) and night (22°C) temperature range occurred in all experiments. Both LW duration and assessment time were always significant, with a significant interaction in 12 of 14 experiments. Web blight severity was positively correlated with increased LW duration. Disease incidence was highest mostly with 20 h LW, which sometimes was not different from 16 and 12 h LW. Eight or fewer hours LW usually resulted in disease incidence not different from 0. When data from all experiments were combined, estimates of maximum disease linearly increased with increased hours LW and hours RH > 75%.

Pycnidial development and pycnidiospore germination of Botryosphaeriaceae species as influenced by temperature

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Phytopathology 101:S39

Two botryosphaeriaceous grapevine pathogens ( Diplodia seriata and *Lasiodiplodia theobromae*) were grown at eight different temperatures to determine the effect on pycnidial development and subsequent pycnidiospore germination. Pycnidial formation and spore development were evaluated at 48 hour intervals beginning seven days after inoculation. Germination efficiency was then assessed for pycnidiospores harvested from these developed pycnidia. Pycnidial formation and pycnidiospore development were significantly affected by temperature. *D. seriata* pycnidia and pycnidiospores developed more quickly than those of *L. theobromae* at all temperatures. Optimal temperatures for this development ranged between 20 and 30°C for *D. seriata* and 25°C for *L. theobromae*. After two hours of incubation at 25°C, germination success was greater for pycnidiospores formed at 15 and 20°C for *D. seriata* and at 20°C for *L. theobromae*. Overall spore germination rates were higher in *L. theobromae* than in *D. seriata*, and highest in spores formed at 30°C for both species. Results of this study show that these species were capable of producing pycnidia and mature pycnidiospores under a broad range of conditions and that spores produced at all temperatures were capable of subsequent germination. These results give an indication of the environmental conditions that influence the epidemiology, survival and reproduction mechanisms in these species, and may assist in generating forecasting models.

Ecology of Bacillus amyloliquefaciens on wheat florets in relation to biological control of Fusarium

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Phytopathology 101:S39

The TrigoCor strain of *Bacillus amyloliquefaciens* is a promising biological control agent (BCA) for Fusarium head blight (FHB) of wheat, caused by the fungus *Fusarium graminearum*. We are using TrigoCor as a model to understand why it, like many BCAs, provides consistent FHB control in the greenhouse but not in the field. Using dilution plating, we periodically quantified *Bacillus* populations from wheat heads for 14d post-*Bacillus* application in the greenhouse and in two NY fields. Although *Bacillus* populations were fairly stable on heads in both environments, the population level in the greenhouse (10^6 CFUs/head) was significantly higher than in the field in 2009 (10^5-10^6 CFUs/head) and 2008 (10^5-10^6 CFUs/head). In 2010 trials, we increased the amount of *Bacillus* applied per head, resulting in levels comparable to those recovered from the greenhouse. Despite these high *Bacillus* levels, there was still insufficient FHB control, suggesting that population levels alone do not explain biocontrol. We used LC to measure levels of key *Bacillus*-produced antifungal compounds on wheat heads from both greenhouse and 2010 NY field trials. Although the levels of compounds on heads decreased rapidly by 3d post-application in both environments, the quantity per head was significantly higher in the greenhouse than in the field. Inadequate levels of antifungal metabolites on wheat heads, perhaps in

other pests and pathogens of national concern. The impacts of the Plant Biosecurity Short course are over 36 graduate students, 200 undergraduate, and 50 K-12 students have received training in surveillance, detection, identification, and response to high consequence plant pathogens.

Using pathogen dispersal characteristics to improve biological control of Canada thistle with the rust fungus *Puccinia punctiformis*

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Phytopathology 101:S39

The noxious weed Canada thistle, *Cirsium arvense*, causes extensive problems in pasture, field crops and natural areas worldwide. The rust fungus *Puccinia punctiformis* is a promising biological control agent that reduces Canada thistle infestations through fatal, systemic infections. Successful augmentative biological control with *P. punctiformis* aims to establish severe, self-sustaining epidemics with natural spread of the pathogen. To better understand and predict the behavior of epidemics under different conditions and improve biological control, we performed a series of experiments to evaluate dispersal characteristics of the various *P. punctiformis* spore types. Dispersal gradients, measured by capturing spores at varying distances from a point source, were different between the two spore types. Specifically, teliospores exhibited a steeper gradient than urediniospores. Comparisons of spore terminal velocities in a particle settling tower indicated that teliospores had a mean fall velocity almost three times that of urediniospores. The timing, effect of environmental conditions, and quantity of spore release was also investigated with spore trapping from diseased field patches. The significance of all these dispersal characteristics will be discussed in the context of applying pathogen predictive modeling to improve weed biological control.

Ecological and ecological effects on inundate release *Trichogramma dendrolimi* to control Asian corn borer in northern China

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Phytopathology 101:S39

*Trichogramma dendrolimi* has been used over 40 years as a prevalent method for control Asian corn borer (*Ostrinia farnacalis*) population on the north part of china. A large scale inundate release on long-term basis resulted in the population suppression of the corn borer under the economical threshold in the mountainous region and the hilly area as well as the flat area with rich vegetation. Stabilization period can be maintained for 4–6 years if the release last over 10 years. The suppression of the corn borer population under the economical threshold in plain area also be attained by means of the cover release for the whole area of the dispersion radius of Asian corn borer (4 km) in large scale and in consecutive year release. Tactics on control corn borer in secondary generation occurrence area of corn borer is the combination of inundate release to control first generation of corn borer and inoculate release to control second generation of corn borer. Release time was minimized to one time by mixing the wasps which was on the different develop stage to one egg-card to make the adult emergence successional to cover whole egg stage period of the pest.

Characterization of Poculum sp. isolated from warm-season turfgrass in Florida

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Phytopathology 101:S39

Fungi were isolated from warm-season turfgrass in Florida with symptoms that differed from typical dollar spot, in that infection centers were larger in diameter, and affected grass had a distinctly lighter color. Isolates produced cultures with distinct substratal stroma morphology, size (0.5 – 2.0 mm), and pigmentation different than the dollar spot fungus, Sclerotinia homoeocarpa. Three loci commonly used for fungal speciation were subjected to neighbor joining and parsimony analyses and included ITS, β-tubulin, and EF-1α. Strongly supported lineages in the phylogenies were consistent with groupings of isolates based on morphological features. Seven out of eight isolates shared higher sequence identity to *P. henningsianum* (93.1%) than to *S. homoeocarpa* (84.7%), but one of the eight had a higher identity to *S. homoeocarpa* (93.8%) compared to *P. henningsianum* (86.9%). These data indicate the isolates belong to a previously undescribed species of *Poculum* closely related to *S. homoeocarpa* and *P. henningsianum* in the Rutsstroemiaeae.
conjunction with low *Bacillus* population levels, may limit FHB control in field situations.

Standardization of protocols to test wheat (*Triticum aestivum* L.) for reaction to blast in a biocontainment laboratory

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Phytopathology 101:S40

Growth medium, spore age, and inoculum density are essential factors for determining host responses to a plant pathogen. The standardization of these factors is important to obtain adequate and reproducible disease assessments. We are testing U.S. wheat cultivars for reaction to the exotic disease blast, caused by the fungus *Magnaporthe oryzae*, in a Biosafety level 3 lab. Although several protocols have been published, it was necessary to adapt those to a biocontainment environment. Four culture media (potato dextrose, V8, oat meal, and corn meal agar), three spore ages (7, 14, and 21 days), and two levels of spore hydration (non-dried and dried) were used to study their effect on appressoria formation by isolate T-25. Over 99% of hydrated and then dried spores did not germinate regardless of growth medium or age, and spores from 14- and 21-day-old cultures had low germinability. The preferred method for production of large amounts of infective spores was the use of 7-day-old cultures from V8 or oat meal agar. Hydrated conidia and conidia that had been dried for 1 min., 15 min., 30 min., or 1 hr, before being rehydrated, were tested for infectivity. In general, dried, and then rehydrated conidia lost their germinability and did not infect wheat. The optimum inoculum density and time of inoculation were determined on three cultivars showing different levels of susceptibility. The preferred inoculum density was 20,000 spores per ml with an inoculation volume of 1.0 ml per wheat head.

Extracellular trapping of bacteria in plant defense responses: Dynamics and specificity

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Phytopathology 101:S40

Major diseases infecting crops are caused by soilborne pathogens. Chemical control of these pathogens has been severely restricted due to health and environmental concerns. A natural plant-based extracellular material provides effective defense for plant root tips which house root meristems. This complex consists of polysaccharides and proteins that have been shown to adhere to and immobilize soilborne pathogens, yet the underlying mechanism has remained obscure. Vertebrate defense has been found to involve production of ‘extracellular traps’ by cells which use extracellular DNA (exDNA) to immobilize and kill bacteria, fungi, and protozoa. Protection of root tips occurs by a similar process. Root tips of most plants produce thousands of healthy ‘border’ cells that protect root meristems from injury and infection by immobilizing toxins, bacteria, fungi, and nematodes. Border cells appear to employ exDNA in a manner directly analogous to that of white blood cells. This new discovery will make it feasible to explore the production, function, and use of plant pathogens by non-treated infected plants. Only some few hyphae were found in the cortex for both K-phi treated and control plants. However, at 4 dpi the vascular region was highly colonized mainly within the phloem, whereas the presence of pathogen in cortex was still low. At 8 dpi the whole root system was colonized; the cortex tissue was highly infected and severely disrupted. At that time Oogonia were found. Nonetheless, in plants treated with K-phi, *P. plurivora* was mainly confined to the vascular region and only some few hyphae were found in the cortex. No mortality was recorded for K-phi treated plants, whereas non-treated infected plants showed mortality up to 66%. We conclude that once the pathogen access the roots it grows directly towards the phloem (sugar sink) and from there colonizes the whole tissue later on. When K-phi is sprayed on leaves it is transported to the whole plant through phloem cells. At the time the pathogen reaches the phloem, K-phi acts as a local fungicide killing the pathogen and avoiding further colonization.

Potassium phosphate protects European beech (*Fagus sylvatica*) seedlings against *Phytophthora plurivora*

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Phytopathology 101:S40

Potassium phosphate (K-phi), a salt of phosphoric acid, was recently described to have an ability to protect plants against certain *Phytophthora* species. In this work, we describe the effect of 3 concentrations of K-phi treatment on beech seedlings infected with *P. plurivora*, an aggressive root pathogen. Leaves were sprayed with 0.5; 0.25 and 0.05% K-phi four days before roots were inoculated with 5 × 10^5 zoospores. Non-inoculated control plants showed a slightly decrease in water uptake and photosynthesis with increasing K-phi concentration probably due to local leaf necrosis after the treatment. However, no mortality was observed. In contrast, all infected plants, including those treated with 0.05; 0.25% K-phi exhibited a strong decrease in water uptake and CO2-assimilation rates, as well as a strong wilting of leaves. About 66% of these plants died after 8 days of infection. Remarkably, all inoculated seedlings treated with 0.5% K-phi did not show any mortality, any wilting symptom and all physiological parameters were comparable to control plants. Furthermore, *P. plurivora* was successfully re-isolated from infected roots of non-treated and K-phi treated seedlings. These results clearly show that K-phi at 0.5% can protect beech seedlings from infection by *P. plurivora*, but not in lower concentrations. Nevertheless, it is still ambiguous whether K-phi acts as a local fungicide or as a resistance inducer. Experiments are ongoing to clarify this question.

Management of aflatoxin contamination of corn in Oklahoma

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Phytopathology 101:S40

Aflatoxin contamination caused by *Aspergillus flavus* is an important constraint to corn production. Afla-Guard, a toxigenic strain of *A. flavus*, was evaluated for control of aflatoxin contamination at various application timings, rates, and formulations. In the timing study, the granular formulation was applied at the V9 stage interval, from V3 to V7, and the wettable granule (WG) formulation (25 g/ha) was applied at VT. In the rate study, granules were applied from 5.6 to 22.4 kg/ha, and the WG formulation at 25 and 50 g/ha at VT. Plots were inoculated with a toxicogenic strain 7-d after VT. AflaGuard control exceeded 50% for all treatments, but treatments applied from V9 to VT had higher levels of ear rot (80 to 100%) than the non-treated check (P < 0.05). Aflatoxin levels were low, exceeding 1100 ppb for the non-treated check in both trials. Treatments reduced aflatoxin levels from 59 to 87% for granules applied at V9 to VT (P = 0.05).
but not at V3 or V6. When applied at VT, all rates of each formulation reduced aflatoxin levels by 64–85% (P = 0.05). However, increasing rates of either formulation did not further reduce aflatoxin level. Kernels with bright greenish yellow fluorescence ranged from 3–5% and did not differ among treatments. However, kernel fluorescence was positively correlated (P = 0.05) with ear rot incidence (r = 0.32 to 0.41). Treatment effects on yield were not significant. Afla-Guard was effective in reducing aflatoxin contamination in corn, but did not reduce levels below 20 ppb.

Residual efficacy of fungicides for brown patch management

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Phytopathology 101:S41

Brown patch, caused by Rhizoctonia solani, is a serious disease of creeping bentgrass and golf course turf. In many parts of the Midwestern U.S., disease-related damage is avoided by applying fungicides preventively during periods of hot humid weather, at 14- to 28-day intervals. Fungicides sometimes fail to provide adequate control for the entire application interval, suggesting that chemical protection is depleted sooner than expected. Our objective was to investigate the nature of the depletion of fungicide protection from turf. A bioassay was conducted with five fungicides (azoxystrobin, flutolanil, metconazole, polyxim D, and pyraclostrobin) applied to field plots of creeping bentgrass maintained at fairway height. Samples (4.25 inch diameter cup-cutter plugs) were collected periodically (0, 3, 7, 10, 14, 17, and 21 days after treatment), inoculated with sorghum seed culture of R. solani, and incubated in a controlled environment for 48 hrs. For each sample date, the extent of fungicide protection was determined by measuring diameters of symptomatic turf on treated and untreated turf plugs. Although the shape of the depletion curves differed among fungicides, in general, protection declined rapidly 7–14 days after treatments were applied. Understanding the length of time that effective concentrations of fungicides remain within the turf may improve scheduling fungicide applications for brown patch control.

In silico simulation of massively parallel sequencing as a diagnostic tool for bacterial phytopathogens

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Phytopathology 101:S41

Increasing importation of commodities from countries abroad increases the risk of introduction of exotic plant pathogens. Although individual pathogen assays are available, current screening methods have limited ability to detect multiple plant pathogens concurrently. The advent of massively parallel sequencing (MPS) technology allows for the creation of a single assay to detect simultaneously, any and all microbes in a sample, including pathogens that have been genetically modified. In this project, we created bioinformatic pipelines, streamlined PC programs, for mock sample databases generation used in simulating 454 runs, query “probe” design and BLAST searches. Pathogen specific queries, ranging in lengths from 20 nt to 140 nt, were created for detection of bacterial select agents Xylella fastidiosa 9a5c, Xanthomonas oryzae, and Ralstonia solanacearum race 3 biovar2, as well as for Candidatus Liberibacter asiaticus (not a select agent). Bioinformatic pipelines generated between 20 to over 6000 unique queries for each target bacterium. The query sets were used to BLAST mock sample databases with one host for all pathogen sequences at various ratios. All four bacterial pathogens were readily detectable in silico, suggesting that MPS technology has advantages of existing pathogen detection assays. This research merges bioinformatics and plant pathology for addressing national security needs in the agriculture industry.

The occurrence of late blight in 2010 following the 2009 epidemic

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Phytopathology 101:S41

Late blight caused by Phytophthora infestans (Mont.) de Bary is one of the most destructive diseases of potato and tomato worldwide. Late blight epidemics in the United States in 2009 were severe due to widespread inoculum and weather that was conducive to disease. This study attempted to determine the genotype composition of late blight in the following year’s population. A total of 55 isolates of P. infestans were collected from major crop production areas in the U.S. during 2010. Isolates were characterized by their pathogenicity, mating type, and in vitro metalaxyl sensitivity. They were also subjected to molecular genotyping. Before 2007, isolates collected from potato and tomato crops were mainly the US-8 or US-11 clonal lineages, respectively. However, P. infestans populations in the U.S.A. underwent a significant genetic shift in 2007–2009; isolates with unique genotypes and epidemiological parameters including increased aggressiveness were detected in Florida and throughout the northeastern region of the U.S. Summer 2010 was one of the hottest and driest on record for much of the south and east. Although there were far fewer outbreaks in 2010, four of these novel genotypes were again identified and caused damage in tomato and potato crops.

Characterization of the fungal community in the tomato phyllosphere

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Phytopathology 101:S41

While much is known about the diversity and activity of foliar pathogens, much less is known about fungal populations inhabiting healthy leaves. In this study we investigated fungal community composition in the tomato phyllosphere across four cultivars, three tissue types, and two sampling times. Fungi were isolated from healthy leaves and fruit and categorized using amplified ribosomal DNA restriction analysis (ARDRA) of the ribosomal internal transcribed spacer (ITS) region. Isolates representing each unique ARDRA profile were then selected and their ITS sequences sequenced. From a collection of 273 isolates, 30 distinct genomovars were observed, though only five of these individually represented more than 5% of the collection. These five included species of Cladosporium, Mucor, Epicoccum, and Alternaria. None of these predominant groups showed any significant variation by cultivar or field position. However, Epicoccum and Mucor were more abundant at the later sampling time, and one group of Mucor was only found on mature leaves and fruit at the second sampling time. The activities of the isolates are currently being investigated in greenhouse bioassays for pathogenicity and biocontrol activity.

Nutritional requirements and possible alternate hosts of Xylella fastidiosa that causes pear leaf scorch in Taiwan

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Phytopathology 101:S41

Xylella fastidiosa pear leaf scorch strain (XF-PLS) infects Hengshen pear (Pyrus pyrifolia) and causes leaf scorch and eventual death of the infected trees. Recent study using randomly amplified polymorphic DNA (RAPD) revealed that XF-PLS was genetically distinct from other X. fastidiosa strains, suggesting XF-PLS may harbor unique genetic factors associated with the pathogenicity to Asian pear. To better characterize XF-PLS, its nutritional requirements and possible alternate hosts were conducted by single ingredient elimination from a PD2-based culture medium. Results showed that XF-PLS could grow in a medium without hemin chloride or trisodium citrate dehydrate, whereas it essentially required magnesium sulfate, disodium succinate, and bovine albumin serum for growth. Alternate hosts for XF-PLS were checked by stab-inoculation of ca. 3 x 10^6 cfu/ml bacterial suspension into the stem of tested plants. Inoculated pear plants were kept in a greenhouse at 28°C. Petioles above the inoculation site were harvested every 3 weeks post inoculation for 3 months and were thin-sliced in PD2 broth for bacterial recovery or ground in SCPAP buffer for PCR detection with XF-PLS specific primers PLS-F and PLS-R. Based on the amplification of a 416 bp DNA fragment by PCR and the recovery of XF-PLS colonies on PD2 agar medium, XF-PLS could multiply and migrate up in the vascular tissues of periwinkle and tobacco, two previously reported alternate hosts of Pierce’s disease strain.

Comparison of “Candidatus Liberibacter asiaticus” populations from Brazil, China, and U.S. at two non-related genomic loci

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Phytopathology 101:S41

Huanglongbing (HLB, yellow shoot disease) is a highly destructive disease in citrus production worldwide. “Candidatus Liberibacter asiaticus” is associated with HLB. Information about population diversity of “Ca. L. asiaticus” from
different geographical regions is important for HLB epidemiological research and disease control. In this study, DNA samples of "Ca. L. asiaticus" from Brazil, China, and U.S. were collected. Variation among bacterial population was evaluated using sequences at two non-related genomic loci, CLIBASIA_01645 and CLIBASIA_05610. The former has a hypervariable region with different tandem repeat numbers (TRNs) and the latter has single nucleotide polymorphisms (SNPs). At the CLIBASIA_01645 locus, the Brazil population is predominated by TRN=10 strain, contrasting to the U.S. population with TRN<10 strains being predominant and the China population with highly heterogeneous TRN genotypes, as previously reported (Phytopathology 100:567-572). At the CLIBASIA_05610 locus, all studied China strains, Brazil strains and the TRN=10 U.S. strains were identical, whereas the TRN<10 U.S. strains have unique substitution at 9 positions. By combining the results from these two different molecular markers, it is concluded that 1) China and U.S. populations of "Ca. L. asiaticus" are distinct; 2) Florida has two different "Ca. L. asiaticus" populations; and 3) the Brazil population of "Ca. L. asiaticus" was unique and highly homogeneous.

A highly sensitive and robust single-tube nested PCR assay for the detection of Pineapple mealybug wilt associated virus (PMWaV-2) K. K. DEY (1), W. Borth (1), M. Melzer (1), D. Sether (1), J. Hu (1) (1) University of Hawaii, Honolulu, HI, U.S.A. Phytopathology 101:S42

An assay was developed for the detection of Pineapple mealybug wilt associated virus-2 (PMWaV-2), which is an important factor in the etiology of mealybug wilt of pineapple. The assay combines reverse transcription of RNA isolated from pineapple with a specific and very sensitive, single-closed-tube nested polymerase chain reaction (PCR). The external and nested primer pairs amplified a segment of the coat protein gene of the virus. These external primers were designed to anneal at higher temperatures than the nested primers to prevent primer competition in consecutive amplification reactions. To further reduce potential competition, the external primers were used at one-thousandth the concentration of the nested PCR. The specificity and sensitivity of this assay are much greater than PCR procedures using only a single primer-pair. A Tagman® probe was designed for use in quantitative PCR to directly detect and quantify the PCR amplification products in a single tube assay. The advantages of the single-tube assays using both conventional and quantitative PCR are reduced handling time and prevention of cross contamination compared to regular nested PCR in which the reactions are carried out in two separate tubes. The new assay will be used to study the mechanisms of virus-plant-insect interactions.

Identification of fungi associated with trunk diseases of grapevine (Vitis vinifera) in Chile G. A. DIAZ (1), B. A. Latorre (1) (1) Pontificia Universidad Catolica de Chile, Santiago, CHILE Phytopathology 101:S42

Grapevine trunk diseases have become prevalent in Chile. Symptoms include internal and external necrosis, radial discoloration, bark cracking (HN) and/or soft necrotic (SN) tissue, vascular streaks (VS) and line necrosis (LN) at the margin of necrotic tissue. Shoot stunting, leaf malformation and shoot stunting have also been observed. A total of 413 trunk samples obtained during surveys of central Chile (extended near 900 kilometers along a north to south axis) between 2009 and 2011 were plated on PDA plus tetraacycline, streptomycin, ampicillin and vancomycin to isolate 14 different fungi. Cultural and molecular characteristics were identified and/or molecularly using the ITS1-5.8S-ITS2 of rDNA. Phaeomoniella chlamydospora was most frequently isolated and along with Diplodia seriata was isolated from all four symptom types. Oidium leprosa was isolated from HN, VS and LN. Inocutis jamacensis and Hymenocheaetaceae sp. were isolated from SN, VS and LN. Dothiorea sarmentorum, Neofuscocarum parvum and Seimatosporis sp. were only isolated from HN. Cryptococcus sp. and Cryptosporium sp. were only isolated from VS. Experiments showed that all isolates were pathogenic in grapevines. Based on these results, grapevine trunk diseases appear to be associated with a fungi complex in Chile with Pa. chlamydospora being the most frequently isolated pathogen. Interestingly, Eutypa lata, a commonly found pathogen worldwide, has not been identified in Chile.

Interactions between Fusarium root rot pathogens and Heteroderda glycinis, on soybean roots M. DIAZ-ARIAS (1), G. L. Tylka (1), L. Leandro (1), G. Munkvold (1) (1) Iowa State University, Ames, IA, U.S.A. Phytopathology 101:S42

Fusarium species are ubiquitous in soil and can cause important soybean diseases including root rot. In some cases, root rot can be exacerbated by other pathogens, such as the soybean cyst nematode (SCN). It is unclear whether there are significant interactions between SCN and species of Fusarium causing root rot on soybean, and the impact of these interactions has not been described. In order to determine whether SCN infestation enhances root rot disease in soybean, seedlings of SCN-susceptible and SCN-resistant cultivars were grown in soil infested with Fusarium alone and in combination with SCN under greenhouse conditions. Two isolates from each of eight Fusarium species were tested. There were significant interactions between SCN and some Fusarium isolates causing root rot. Root rot severity, shoot dry weight, and shoot dry weight differed among Fusarium isolates and there were significant interactions between effects of SCN and Fusarium isolates for these variables. Co-inoculation with SCN resulted in greater root rot severity on plants inoculated with six isolates representing five Fusarium species (F. oxysporum, F. graminearum, F. semitectum, F. solani and F. sporotrichioides) on both soybean cultivars. In general, SCN - Fusarium interaction effects for root rot (p = 0.0004), and shoot dry weight (p = 0.02) were greater in the SCN-resistant than in the susceptible cultivar. SCN appears to influence Fusarium root rot, but only for particular Fusarium species.

Distribution and frequency of isolation of Fusarium species associated with soybean roots in Iowa M. DIAZ-ARIAS (1), L. Leandro (1), G. Munkvold (1) (1) Iowa State University, Ames, IA, U.S.A. Phytopathology 101:S42

Species of Fusarium are known as common and widespread fungi that can cause damping-off and root rot diseases in soybean. However, the most important pathogenic species and their overall impact are unclear. In order to characterize the distribution and frequency of Fusarium species associated with soybean roots, we conducted a three-year root survey in 98 Iowa counties. Ten plants were collected from 3 fields per county at both V2-V3 and R3-R4 growth stages. Soybean root pieces were surface sterilized and placed onto a Fusarium selective culture medium. Fusarium colonies were identified to species based on cultural and morphological characteristics. Species identification was confirmed for selected isolates by amplification and sequencing of the translation elongation factor (TEF) gene. Twelve Fusarium species were identified; F. oxysporum, F. solani, F. acuminatum and F. avenacearum were the most frequent and widespread species. Some species (F. semitectum, F. subglutinans, F. virguliforme, and F. poae) were recovered from a low percentage of fields. Most of the species have been reported on soybean before, but some have not previously been associated with soybean roots such as F. subglutinans, F. semitectum and F. sporotrichioides. Species prevalence among fields differed regionally within and between years. Differences in species frequency were found between growth stages; greater species diversity occurred in roots at V2-V3 stages compared to R3-R4 stages.


Our complete genome of the root-knot nematode (RKN) Meloidogyne hapla provides a powerful platform to study plant-metazoan interactions. We are particularly interested in nematode encoded genes that mimic plant regulatory molecules, especially members of the CLE and CEP families of peptide hormones. Using a double-affine Smith-Waterman algorithm we established that M. hapla encodes eight CLEs, nine CEPs and RALF. Unlike the native peptide ligands which are secreted as pro-proteins and require regulatory cleavage, the M. hapla mimics presumably are secreted directly into the host apoplast as active hormones, pointing to a direct role in the parasitic interaction. Alignment of family members has revealed striking sequence diversity among M. hapla CLEs and predicts both A and B types documented in model plants. Logplot analysis of the M. hapla CLEs shows a high conservation of residues required for function in native plant peptide hormones. Collectively this suggests that RKN encodes a comprehensive array of plant regulatory functions. As is the case for plant CEPs these M. hapla genes exhibit only limited sequence diversity, although they are clustered in a region of the genome that is hyper variable between wild isolates. Transcription of the nematode mimics was confirmed by qPCR, and using bioassay we examined the effect of the nematode peptides on roots. These experiments demonstrated dose dependent inhibition of growth, thus establishing physiological relevance.

Management of onion purple blotch with bioformulations and fungicides D. DINAKARAN (1), G. Gajendran (1), S. Mohan Kumar (2), G. Karthikeyan (2), S. Mathiyazhagan (1), S. Thiruvadumalib (1), V. Jayabal (1) (1) Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, INDIA; (2) Tamil Nadu Agricultural University, Coimbatore, INDIA Phytopathology 101:S42
Purple blotch [Alternaria porri (Ellis) Neerg.] is a serious disease of onion causing up to 30 per cent yield loss and under favourable conditions causes total crop failure. For the management of purple blotch seven fungicides, two neem formulations and four bioagents were evaluated both in vitro and in vivo conditions. Under in vitro evaluation, solid and liquid media of PDA were poisoned with various concentrations of fungicides and neem products and for antagonistic organisms dual culture technique was adopted. The fungicides viz., tebuconazole, propiconazole and hexaconazole were effective in complete inhibition of A. porri even at 50 ppm level. In pot culture, foliar spray of tebuconazole 0.15% registered the least severity of purple blotch (41.8%) followed by hexaconazole 0.1% and propiconazole 0.1% as against 85.6% in untreated check. Similar trend was observed in field experiment with tebuconazole reducing the least severity of purple blotch (26.7%) followed by mancozeb, propiconazole and hexaconazole. Among the bioformulations, azadirachtin 1% formulation at 0.2% registered the lowest disease severity of 41.1% followed by azadirachtin 0.03% formulation at 0.2% with 43.4% and PGPR (TNAU formulation) at 0.5% with 47.8% as against untreated check with 64.4%. The highest bulb yield of 13.1 t/ha was recorded in tebuconazole followed by mancozeb (12.8 t/ha), propiconazole (12.7 t/ha) and hexaconazole (12.6 t/ha) which were on par. The untreated check recorded the lowest bulb yield of 8.8 t/ha.

Genetic diversity and characterization of geographic distribution and of Begomovirus in Yunnan, China

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Phytopathology 101:S43

Begomoviruses is a group of circular single-stranded DNA viruses that caused economically significant diseases in tropic and subtropics area. The present study describes the characterization of distribution and genetic diversity of begomoviruses in Yunnan province between 2002 and 2008. A total of 1370 samples were collected from 4 different regions of begomoviruses in Yunnan, 42 full-length begomoviruses genome sequences have been amplified by PCR or RCA. Sequence analysis revealed that 42 isolates were belonged to 20 species of the genus Begomovirus, including 10 new report species globally, 7 new report species and 3 recorded species. Base on the different components of viral genome, four phenotypic groups of begomoviruses were identified in Yunnan: (1) single DNA-A component; (2) DNA-A and DNA-B component; (3) DNA-A and betasatellite component; (4) DNA-A, betasatellites and alphastellites component; According to survey about begomoviruses regions, there are four heavily endemic areas with begomoviruses: (1) the valley of Golden Sand River in north of Yunnan; (2) the basin of Yuan River and Red River in south-central of Yunnan; (3) the basin of Lu River and Ruili River in west of Yunnan; (4) the basin of Lancang River in the south of Yunnan. On the basis of the present study, this viral complex has been particularly devastating in different areas of Yunnan and the situation has progressively worsening to a point where this major constraint on the production of many crops in Yunnan province, China.

Comparative transcriptome analyses of Fusarium osyporum f. sp. cubense

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Phytopathology 101:S43

Fusarium osyporum f. sp. cubense (FOC), the causal agent of Panama disease of banana is a highly destructive and genetically diverse pathogen. Despite its economic importance, genomic information of FOC is poor and no transcriptomic analyses have been reported so far. By using 454 sequencing technology, we generated >2.5 million expressed sequenced tags (ESTs) from four FOC strains representing four different vegetative compatibility Groups and the four described races that infect banana: race 1 (R1), race 2 (R2), subtropical race 4 (SR4) and tropical race (TR4). These ESTs were obtained from libraries prepared from mRNA extracted from three physiological conditions (mycelia, conidia and germinated conidia), which were pooled at a 2:2:1 ratio. Most genes are represented in all libraries, but in silico comparative analyses identified a set of unique ESTs for each race (689 for R1, 974 for R2, 296 for SR4 and 555 for TR4), which constitute excellent candidates for future plant-pathogen interaction studies and functional analyses. In subsequent analyses, a 40x sequencing coverage of FOC (TR4) genomic data from NCBI was assembled using a de novo assembly methodology. Preliminary analyses show a high colinearity of EST and genomic data that significantly contributes to the quality of the assembly. Potential applications of these data will be further discussed.

Characterization of three new isolates and extended experimental host range of Phytophthora capsici in Brazil

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Phytopathology 101:S43

Phytophthora capsici is an important pathogen affecting many host species. During this study the association of P. capsici with new diseases on snap bean pods, gherkin (Cucumis anguria L.) and strawberry fruits in Brazil. In addition, we evaluated a set of plant species as potential hosts of P. capsici at both seedling and fruit stages. Test plants (87 accessions of 68 species) were evaluated for their reaction to four isolates (obtained from snap bean, Capsicum, gherkin, and strawberry fruits). Seedlings were inoculated by placing 3 mL of the zoospor suspension (2 × 104 zoospores/mL) into the soil around the seedling. The A. c. anuum cultivars ’Kedal’ and ‘SCM-934’ were used as susceptible and resistant controls, respectively. Fruits were inoculated by placing mycelial plugs on the fruits after toothpick injury. All isolates were identified as P. capsici according to their morphology and by the sequences of its ITS regions. At the seedling stage, 36% of the accessions were found to be susceptible in contrast with 85% in the fruit evaluation. New non-solanaceous and non-solanaceous hosts were found in both assays. This updated P. capsici host list includes new reports for Brazil such as strawberry, gherkin, tobacco, snap and common bean plants as well as apple and pear fruits.

Increasing the sensitivity of PCR for the detection of foodborne pathogens in fresh produce

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Phytopathology 101:S43

Contamination of fresh produce by human pathogens, such as E. coli O157:H7 and Salmonella, is a serious threat to human health and to the fresh produce industry. Early detection of the pathogens is critical in ensuring safety of fresh produce, from preharvest certification of field lots to quality control of the final market product. The aim of this study was to evaluate the effect, on PCR sensitivity, of adding a 5’ AT-rich overhanging sequence (flap) to the design of primers specific for the detection of E. coli O157:H7 and Salmonella. Specific primers targeting the rfbE O157 and invA genes for E. coli O157:H7 and Salmonella, respectively, were synthesized with or without a 12-bp 5’TAT-rich overhanging sequence. PCR sensitivity assays were conducted using purified E. coli O157:H7 and Salmonella genomic DNA, crude cell lysates, and genomic DNA/crude cell lysates spiked with DNA extracted from surface wash water from tomato and jalapeno peppers. PCR amplicons were eluted and quantified using a nano drop spectrophotometer. Amplicon band intensities were significantly higher when primers contained the flap, and the yield of PCR amplification for Salmonella and E. coli O157:H7 was increased by 20 and 23%, respectively. Improvement in the efficiency of PCR detection has potential applications in routine food safety monitoring, foodborne disease epidemiology and management, and in biosecurity and microbial forensics applications, as fewer target pathogens can be detected in less time.

Citrus-CTV molecular interactions: What is the host side of the story?

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Phytopathology 101:S43

Tristeza caused by Citrus tristeza virus (CTV) is a unique example where cross protection (CP) has been successfully used. However, in most instances, CP has broken down over time. Lack of knowledge of the molecular regulatory mechanisms of CP is the major factor and hampers efforts to improve and increase sustainability of CP. There are multiple anecdotal reports on the virus strains involved in CP, but molecular events in citrus that result in CP are unknown. Deep sequencing (Illumina) analysis of small interfering RNAs (siRNAs) extracted from sour orange (SO) severely affected by seedling yellows (SY) and from the cross protected SO showed atypical accumulation of virus-derived siRNAs in the 3’ end of the CTV genome. Contigs from these datasets identified sequences highly homologous to the 282 Kb Ctv resistance locus of Poncirus trifoliata, providing strong evidence that this region is involved in the siRNA pathways. Similarly, a total of 50 miRNA families showed significant sequence presence, of which 8 miRNA families showed differential expression. Analysis of Citrus mRNAS (EST) targets for these miRNAs in the cross protected SO plant and the SO showing strong SY and healthy untreated SO control show that there are 433 targets that are common to both the treated plants (absent in control), 873 targets that
are only in the cross protected SO plant and 753 targets in the SO showing strong SY, suggesting dynamic rewiring of the miRNA-mRNA interaction network.

**Potato virus and phytoplasma diseases in Yunnan, China**

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Phytopathology 101:S44

In recent years, virus and phytoplasma diseases are the important constraints to potato production in Yunnan, China. Field surveys were conducted from 2006 to 2010. A total of 415 potato plant samples, including 302 samples showing virus-like symptoms and 113 samples showing phytoplasma-like symptoms, were collected from potato-growing regions in Yunnan. A total of 184 seed potato samples, including 45 potato seedling, were collected from the local seed potato-produced bases. All the samples were tested using the DAS-ELISA kits purchased from Agdia(US). The results showed the overall virus infection rate was as high as 65.8%. Infections by Potato virus S (PVS), Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus A, Potato virus X, Potato virus M were 233 (38.9% of total), 97 (16.2%), 92 (15.4%), 56 (9.3%), 52 (8.7%), 10 (1.7%), respectively. 264 samples were infected with more than one virus. PVS-PLRV was the most frequently detected in the mixed infection (7.5%), followed by PVS-PVY (7.0%). Phytoplasma samples were detected by nested-PCR using the reported primer pairs, and then sequenced. Sequence analysis showed at least three Candidatus phytoplasma species infecting potato in Yunnan, including Candidatus Phytoplasma asteris, Candidatus Phytoplasma trifolii, and Candidatus Phytoplasma fragariae. Among 45 potato seedling tested by RT-PCR using Potato spindle tuber viroid (PSTV)d specific primers, and sequence analysis, 8 samples were infected by PSTVd.

**An elution-independent collection device for rapid sampling of microorganisms and nucleic acids for PCR assays**

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Phytopathology 101:S44

A novel elution-independent collection device (EICD), Oklahoma State University patent pending reference number 2010.26, was designed for rapid collection of microorganisms and recovery of nucleic acids. The easy-to-implement EICD collects fluid samples by contact and lateral flow. Minute pieces (1.2 mm diameter) of a built-in soluble element dissolve directly in commercial PCR mixtures without an intermediate elution step, thereby streamlining PCR based assays. More than 27 different materials from 5 different manufacturers were assessed for one-step RT-PCR assays (without an intermediate RNA extraction step). The resulting EICD prototype is effective i.e. on sap from tobacco plants infected with Tobacco mosaic virus (TMV) and bacterial suspension (Erwinia tracheiphila), as well as whole ground insects (Liposcelis brunnea). All samples were ground in PBST buffer, and bacterial and insect samples were microwaved 30 seconds before loading. Control treatments consisted of NA extracted with commercial kits. Positive amplifications of each specific target were observed regardless of whether extracted materials were from plants, insects, soils, etc. The EICD prototype were ready for PCR processing within 3 minutes, far less than the 10-30 minutes required using commercially available kits. This EICD can be a rapid sampling choice in molecular-clinical diagnostic applications for medical, veterinary, plant health biosecurity, forensics and foods quality.

**Aflatoxin producing potential and community structure of Aspergillus section Flavi in almond orchards of the Central Valley of California**

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Phytopathology 101:S44

Aflatoxins are highly carcinogenic secondary metabolites produced by Aspergillus section Flavi. Several nut crops including almonds, pistachios, and walnuts are affected by aflatoxin contamination. The aflatoxin-producing ability of the aflatoxin soil communities constitutes a major risk factor for aflatoxin contamination in almond orchards. Over 2,100 Aspergillus isolates from 28 almond producing orchards located in the northern, central, and southern Central Valley (California) were isolated from soil samples during 2007 to 2010. The aflatoxin-producing potential of over 800 A. flavus L-strain isolates was also determined. Results indicate that A. flavus L-strain was the most common, followed by A. parasiticus. Although the incidence of the highly aflatoxin-producing A. flavus L-strain was low (5%) in 2007, it increased to 28% in 2010, which could increase the risk of aflatoxin contamination in almond orchards and other nut crops. Preliminary data indicate that the aflatoxin-producing potential of the L-strains increased from 63% of toxigenic isolates in 2007 to 80% in 2008. The L-strains isolates from the northern orchards showed the highest aflatoxin-producing potential in both years. Incidence of atoxigenic A. flavus communities decreased in all regions of the valley from 2007 to 2008. The incidences of aflatoxin-producing isolates in the A. flavus communities in the orchards pose a risk of aflatoxin contamination of nut crops in California. The almond industry has taken a number of measures pre- and post-harvest to assure control and compliance with aflatoxin standards. These measures include: 1) Good agricultural practices like insect pest management and product handling; and 2) Sorting of insect damaged kernels.

**Genetic diversity of Candidatus Liberibacter asiaticus strains from Thailand based on DnaA and TuB genes**

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Phytopathology 101:S44

Huanglongbing (HLB) is considered as one of the most destructive diseases of citrus in the world because it causes rapid decline of infected trees. The HLB widespread throughout Asia and some parts of North and South America is Candidatus Liberibacter asiaticus (LAS). Two additional HLB bacteria are Ca. L. americanus (LAM) and Ca. L. africanus (LAF) which are reported only in South America and Africa, respectively. Genetic diversity among LAS strains from Thailand and other countries was investigated by analyzed dnaA and tuB genes of Candidatus Liberibacter asiaticus. Total 45 laseraria and Ca. Liberibacter asiaticus were collected from different hosts and geographical regions. PCR were amplified from total DNA by primers dnaA; 5'-CCCCCTCTCCGCCGCGCAACAT-3', dnaA; 5'-ACTGGCCTGTTGAAA GCCCA-3' for 334 bp of dnaA gene and tuB; 5'-TCTTGGCATGTGATTAGT-3', tuBR; 5'-CGCAGCTTACTCCTACGAAA-3' for 539 bp of tuB gene. PCR products were sequenced and analyzed. Phylogenetic tree of dnaA sequences showed most of Thai strains were difference from strains of China and Brazil. Three Thai strains were closely related to strains from Asian countries such as Vietnam, Indonesia, the Philippines and Taiwan. 19 other Thai strains were separated in 4 other groups. Phylogenetic tree of tuB gene showed more conserved among strains from all regions except some strain of Thai, China and Brazil which were diverse. The results indicated that dnaA gene was correlated to geographical regions whereas tuB gene was less genetic diversity.

**The dynamics of ABA biosynthesis by Cercopora zea-maydis**

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Phytopathology 101:S44

Gray leaf spot, caused by Cercopora zea-maydis is an economically important yield-limiting foliar disease of maize. During infection, maize leaves infected with the foliar pathogen Cercospora zeamays lose their turgor, aperture and host response to pathogen attack. Interestingly, we recently observed that C. zeae-maydis produces ABA in culture, suggesting a possible function in pathogenesis, given that C. zeae-maydis infect the host through the stomata. However, the role of fungal ABA biosynthesis during infection is poorly understood. Through degenerate PCR, we have identified a putative ortholog of the Botrytis cinerea ABA3 gene, which is being disrupted by split marker homologous recombination for functional characterization. To elucidate the importance of ABA in fungal pathogenesis, the regulation of ABA biosynthesis in response to metabolic and environmental cues is being determined by direct LC-MS measurement of ABA production and expression of the putative ABA3 gene by qPCR. This work will shed light on the regulation of ABA biosynthesis at the molecular level and serve as a foothold for further elucidation of the function of ABA produced by fungi during pathogenesis.

**Distribution of Arabis mosaic virus (ArMV) on grapevines and roses in Western and Eastern Azarbaijan Provinces, Iran**

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Phytopathology 101:S44

Arabis mosaic virus (ArMV) belongs to the plant virus genus Nepovirus in the family Secoviridae. ArMV is transmitted by several species of the nematode vector in genus Xiphinema, and has a very wide natural host range in crop plants and ornamentals. The objective of this study was to determine the distribution of ArMV through the vineyards and rose plantations in Western and Eastern Azarbaijan Provinces of Iran. To achieve this aim a total number
haplotypes identified based on chromosomal repeats in over 600 isolates. Isolates with further biogeographical stratification. This study demonstrates that ArMV was distributed through the rose plantations and vineyards of 1043 samples including 251 rose and 792 grapevine leaf samples were tested for the presence of ArMV using RT-PCR method. DNA fragments of 440 bp and 519 bp were amplified from the serological positive ArMV samples. To our knowledge this is the first precise study on the ArMV distribution through the different rose plantations and vineyards in several districts of West and East Azerbaidjan Provinces of Iran.

Protect U.S.: Community-based invasive species education for small farmers and the general public


Phytopathology 101:S45

The community invasive species network (www.protectingusnow.org) is concerned with protecting the U.S. from exotic, invasive species. Protect U.S. is a collaborative partnership between the National Plant Diagnostic Network (NPDN), Regional Integrated Pest Management (IPM) Centers, United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-PPQ), National Institute of Food and Agriculture (NIFA), the National Plant board, the Department of Homeland Security (DHS) your local Land Grant University Cooperative Extension Service, and other organizations involved in exotic species extension and regulatory activities. Target audiences for Protect U.S. educational content include small farmers, the general public, and K-12 audiences. Protect U.S. content may be integrated into NPDN First Detector training, but is delivered in a simplified format for audiences not traditionally reached through this program. The Protect U.S. website officially launched in September of 2010, and a national train-the-trainer webinar occurred in February of 2011. By December of 2011, e-learning modules and scripted powerpoints (for educators) will be available for Protect U.S. audiences, including interactive quizzes, games, and certificates of completion. Project outcomes, including the number of learners completing modules, web statistics, webinar feedback, and opportunities for module authorship will be presented.

Biogeographic diversity analysis of Erwinia amylovora using multi-locus variable number of tandem repeats analysis (MLVA)

T. DREO (1), T. H. Smits (2), J. E. Frey (2), M. Ravnikar (1), B. Duffly (3) (1) National Institute of Biology, Ljubljana, SLOVENIA; (2) Agroscope Changins-Wädenswil ACW, Wädenswil, SWITZERLAND; (3) Swiss Federal Research Station, Wadenswil, SWITZERLAND

Phytopathology 101:S45

Fire blight is a chronic, major threat to sustainable pome fruit production. Phytosanitary control strategies have relied on inoculum source assumptions. We applied recent genetic sequencing to develop MLVA for deep resolution of strain genotypes as a tool to enable inoculum reservoir source tracking. Genome-wide DNA repeats were identified starting with the complete sequence of CFBP 1430. Six selected repeats regions of 6-18 bp were amplified in multiplex PCR with labeled primers allowing determination of the number of repeats at each locus with capillary electrophoresis. MLVA analysis was applied to a large number of E. amylovora isolates, representing global and regional diversity of the pathogen. High diversity was observed than in previously employed genotyping methods and more than 92 haplotypes identified based on chromosomal repeats in over 600 isolates. Shannon’s diversity index for individual loci ranged from 0.18 to 0.61. Two large groups with further sub-groupings were recognized among global isolates with further biogeographical stratification. This study demonstrates the potential of MLVA for further development as a tool for epidemiological studies, geographical surveillance, and source-tracking of E. amylovora.

Many bacterial biological control agents produce secondary metabolites that influence plant-microbe interactions. These are often controlled by regulatory networks such as the GacA/S two component system. The genetic stability of GacA/S is influenced by growth conditions; spontaneous Gac mutants often become the majority in nutrient-rich media. Natural populations often contain variants with defective Gac systems. These mutants have a decreased metabolic load but do not displace the wild type. How does natural selection maintain the wild type in the presence of a mutant with enhanced growth? One hypothesis is that Gac mutants are ‘cheaters’ that do not contribute to the public good: favored within groups but selected against between groups. An alternative hypothesis is that Gac mutants have a mutualistic interaction with the wild type: each variant benefits by the presence of the other. Pseudomonas syringae subsp. syringae variants do not produce phenazines, which inhibit pathogens and are critical for biofilm formation. We tested the predictions of these hypotheses by quantifying interactions between the wild type and Gac mutant within growing biofilms. We found that the wild type and Gac mutants interact mutually. Our results suggest that the persistence of alternative Gac phenotypes may be due to the stabilizing role of local frequency-dependent selection in structured environments and may be a conserved strategy which improves overall success.

On-farm research activities to implement methyl bromide alternatives: An area wide initiative update

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Phytopathology 101:S45

On-farm research (OFR) is an important mechanism to enable growers to implement alternatives in strawberry production systems and solve disease and weed management challenges. In 2009 to 2010, three strawberry trials were conducted. For each of the sites, the fumigation material evaluated was based on the need and desire of the specific grower according to farm specific pest complexes. All sites included the use of virtually impermeable films (VIF) to cover the raised beds combined with reduced rates of fumigants. Fumigants used in the strawberry trials included: Methyl Bromide (50% methyl bromide=50% chloropicrin; MB) at 240 lb/A, Pic-Clor 60 shank applied at 188 lb/A and dip applied at 250 lb/A, Inline (60% 1,3-dichloropropene+33.3% chloropicrin) dip applied at 26 gal/A, Telone C-35 shank applied at 294 lb/A, MIDAS (50% methyl iodide+50% chloropicrin) shank applied at 150 lbs/A and metam sodium drip applied at 75.0 gal/A. growers applied using reduced rates under VIF mulch. Products were applied using grower equipment under grower selected conditions with a minimum of 10% of the total acreage fumigated with a MB alternative. In most cases, the OFR was arranged in a RCBD and growers collected harvest data semi-weekly. Alternatives were found to work as well or better than the MB treatment and provided confidence to growers to transition away from MB.

Sensitivity of Magnaporthe grisea to isoprothiolane, iprobenfos and tricyclazole Y. Du (1), K. Li (1), H. Ruan (1), X. Lu (2), X. Yang (1), F. CHEN (1) (1) Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou, PRC PEOPLES REP OF CHINA; (2) Plant Pathology Dept, China Agricultural University, Beijing, PRC PEOPLES REP OF CHINA

Phytopathology 101:S45

In this study, the sensitivity of Magnaporthe grisea to three fungicides was determined by mycelium growth rate method. 82 M. grisea isolates were collected from disease nurseries of various villages and cities in Fujian province, where there was no history of fungicide applications. Baseline sensitivities of M. grisea to isoprothiolane and iprobenfos were established with a mean EC_{50} (effective concentrations for 50% inhibition of mycelial growth) of 2.4369 µg/mL and 6.9168 µg/mL, respectively, which showed an unimodal distribution. 141 intermediate-resistant isolates to isoprothiolane were 14.89%, 81.56% and 47.61 µg/ml, which showed a skewed unimodal distribution and no resistant province, where there was no history of fungicide applications. Baseline sensitivities of M. grisea to isoprothiolane and iprobenfos were established with a mean EC_{50} (effective concentrations for 50% inhibition of mycelial growth) of 2.4369 µg/mL and 6.9168 µg/mL, respectively, which showed an unimodal distribution. 141 intermediate-resistant isolates to isoprothiolane were 14.89%, 81.56% and 47.61 µg/ml, which showed a skewed unimodal distribution and no resistant

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Spontaneous Gac mutants in Pseudomonas syringae control strains: Are they cheaters or mutualists? W. W. Driscoll (1), L. S. Pierson (2), E. A. PIERSKALD (3) (1) Ecology and Evolutionary Biology Department, University of Arizona, Tucson, AZ, U.S.A.; (2) Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, U.S.A.; (3) Department of Horticultural Sciences, Texas A&M University, College Station, TX, U.S.A.

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We applied recent genome sequencing to develop MLVA for deep resolution of strain genotypes as a tool to enable inoculum reservoir source tracking. Genome-wide DNA repeats were identified starting with the complete sequence of CFBP 1430. Six selected repeats regions of 6-18 bp were amplified in multiplex PCR with labeled primers allowing determination of the number of repeats at each locus with capillary electrophoresis. MLVA analysis was applied to a large number of E. amylovora isolates, representing global and regional diversity of the pathogen. High diversity was observed than in previously employed genotyping methods and more than 92 haplotypes identified based on chromosomal repeats in over 600 isolates. Shannon’s diversity index for individual loci ranged from 0.18 to 0.61. Two large groups with further sub-groupings were recognized among global isolates with further biogeographical stratification. This study demonstrates the potential of MLVA for further development as a tool for epidemiological studies, geographical surveillance, and source-tracking of E. amylovora.
Isolates CP98B and CP08C6 were identified as harvest chickpea debris in eastern Washington in 2003, 2008 and 2009. Phytopathology 101:S46 does not consistently induce wilt in Washington State. Further investigation will underpin the exact amino acid changes contributing to phenotype. A yeast recombination method was used to construct an infectious clone and chimeric viruses contained within a yPYES2.1 vector under control of a Sp6 promoter. RNA transcripts generated from the Sp6 promoter were infectious on a range of indicator plants including Nicotiana benthamiana, Nicotiana glutinosa, Nicotiana tabacum, Datura stramonium and Lycopersicon esculentum. Three weeks post inoculation 100% of plants tested positive and sequencing RNA from the infected plants confirmed no cross-contamination had occurred. Both ELISA and real-time PCR testing showed all clones had titres equal to wild-type. The clone of the EU strain can move systemically in Nicotiana glutinosa, Nicotiana tabacum, and distinct mosaics in D. stramonium. Further investigation will underpin the exact amino acid changes contributing to the changes in symptomatology with the aim of directly linking genotype to phenotype.

Clonostachys rhizophaga can delay and reduce emergence of chickpea but does not consistently inhibit wilt in Washington State.

Verticillium dahliae, causal agent of Verticillium wilt, can infect >200 hosts. Isolates from mint and potato usually belong to different vegetative compatibility groups (VCGs) which may result in genetic differentiation among V. dahliae isolates from these hosts. Eighty-six isolates from peppermint, scotch spearmint, and native spearmint and 65 isolates from potato, seed potato, and seed potato tare soil were characterized for VCG, mating-type, and multilocus microsatellite haplotype to determine the relationships among V. dahliae isolates affecting potato and mint. All isolates from mint and potato were mating-type MAT1-2 and belonged to VCG 2B and VCG 4, respectively. Genetic diversity was greater among isolates from potato (Hd = 0.87) than mint (Hd = 0.22) and 88% of mint isolates were of one haplotype. A single haplotype accounted for 93% of isolates from seed potatoes and was sampled from potato in several states. Principal coordinate analysis (PCoA) clustered potato and mint haplotypes into distinct groups and analysis of molecular variance (AMOVA) indicated the two groups were significantly different (P = 0.02). Although genetic differences between VCG 4A and VCG 4B accounted for 53% of the total variation, VCG 4 subgroups from potato were not differentiated using PCoA or AMOVA (P = 0.34). Minimum spanning network analysis suggests V. dahliae isolates from mint and potato are genetically distinct but the potential for gene flow exists between VCG 4A and 4B subgroups.

Progress on Industry Pest Information Platform (ipipe)

Progress on Industry Pest Information Platform (iPIPE) has been able to provide support for the initiative through a project funded by Section 10201 of U.S. Farm Bill. The aim of the project is to create a common architecture for information exchange and to enhance the early detection of exotic plant pests. The iPIPE pilot phase will be part of the NAPFFAST system, an information tool developed by NC State University, PPQ and the information technology company ZedX. The pilot phase includes a soybean technology, including Pseudomonas syringae (southern corn rust) and Clavibacter michiganensis subsp. michiganensis (Goss wilt). ASTA companies submit survey and diagnostic data which is displayed anonymously at a county resolution in the iPIPE portion of NAPFFAST. Registered users of iPIPE can view pest observations in an interactive map and overlay weather data, NAPFFAST risk maps and HYSPLIT atmospheric trajectories. The benefits of iPIPE include an improved information network for tracking emerging and exotic plant pests and a better information for permitting and certification. As the system matures, it is expected to reach out to other key partners including the National Plant Diagnostic Network.

Development of a qPCR assay for quantification of Verticillium dahliae in spinach seed

Verticillium wilt, caused by the soilborne fungus Verticillium dahliae, is an important disease of lettuce and other specialty crops in the Salinas Valley of California. Although spinach is not affected by Verticillium wilt in California, it is infected with Clavibacter michiganensis subsp. michiganensis in the U.S. Pacific Northwest and Europe and planted in Salinas Valley increases inoculum density and potentially introduces exotic strains that may contribute to Verticillium wilt epidemics. A sensitive, rapid and reliable method for quantification of the fungi in seed may help to curtail the spread of V. dahliae via spinach seed. The objective of this research is to develop a qPCR assay to detect and quantify V. dahliae in spinach seed. We employed a combination of molecular techniques and high-throughput sequencing to develop a qPCR assay. Verticillium dahliae, causal agent of Verticillium wilt, can infect >200 hosts. Isolates from mint and potato usually belong to different vegetative compatibility groups (VCGs) which may result in genetic differentiation among V. dahliae isolates from these hosts. Eighty-six isolates from peppermint, scotch spearmint, and native spearmint and 65 isolates from potato, seed potato, and seed potato tare soil were characterized for VCG, mating-type, and multilocus microsatellite haplotype to determine the relationships among V. dahliae isolates affecting potato and mint. All isolates from mint and potato were mating-type MAT1-2 and belonged to VCG 2B and VCG 4, respectively. Genetic diversity was greater among isolates from potato (Hd = 0.87) than mint (Hd = 0.22) and 88% of mint isolates were of one haplotype. A single haplotype accounted for 93% of isolates from seed potatoes and was sampled from potato in several states. Principal coordinate analysis (PCoA) clustered potato and mint haplotypes into distinct groups and analysis of molecular variance (AMOVA) indicated the two groups were significantly different (P = 0.02). Although genetic differences between VCG 4A and VCG 4B accounted for 53% of the total variation, VCG 4 subgroups from potato were not differentiated using PCoA or AMOVA (P = 0.34). Minimum spanning network analysis suggests V. dahliae isolates from mint and potato are genetically distinct but the potential for gene flow exists between VCG 4A and 4B subgroups.


Phytopathology 101:S46

Several years ago, the American Seed Trade Association (ASTA) proposed the Industry Pest Information Platform (iPIPE) to share survey and diagnostic data between industry, government and universities. Recently, USDA-APHIS-PPQ has been able to provide support for the initiative through a project funded by Section 10201 of U.S. Farm Bill. The aim of the project is to create a common architecture for information exchange and to enhance the early detection of exotic plant pests. The iPIPE pilot phase will be part of the NAPFFAST system, an information tool developed by NC State University, PPQ and the information technology company ZedX. The pilot phase includes a soybean technology, including Pseudomonas syringae (southern corn rust) and Clavibacter michiganensis subsp. michiganensis (Goss wilt). ASTA companies submit survey and diagnostic data which is displayed anonymously at a county resolution in the iPIPE portion of NAPFFAST. Registered users of iPIPE can view pest observations in an interactive map and overlay weather data, NAPFFAST risk maps and HYSPLIT atmospheric trajectories. The benefits of iPIPE include an improved information network for tracking emerging and exotic plant pests and a better information for permitting and certification. As the system matures, it is expected to reach out to other key partners including the National Plant Diagnostic Network.

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Potato IPM program: Taking the research to the farm
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Phytopathology 101:S47

Potatoes are the largest agricultural commodity in the State of Maine, generating a total economic value of over $500 million, and producing employment for over 6,000 individuals. The University of Maine Cooperative Extension Potato IPM program is a multidisciplinary project that assists growers in the management of potato pests by providing specific and timely information. Information gathered through multiple sources, including direct observation, traps, weather data, and prediction models, is delivered to potato growers in Maine and around the globe through electronic and standard newsletters, websites, and via telephone message centers. The program generates a total economic value of over $500 million, and producing over 1.5 million data points that help IPM scientists track pest outbreaks and provides potato growers and industry professionals with current information on specific and timely treatments, which can be used to minimize pesticide applications and maximize potato yield and quality. In 2009, the UMaine Cooperative Extension Potato IPM Program produced an estimated $26 million positive impact on the Maine potato industry.

Coat protein expression strategy of oat blue dwarf virus
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(1) USDA ARS, Fargo, ND, U.S.A.
Phytopathology 101:S47

Oat blue dwarf virus (OBDV) was the first marafivirus (family Tymoviridae) to be sequenced and for which an infectious clone has been reported. Although sequence data are now available for multiple marafiviruses, precise details of the expression strategy of these viruses remain undocumented. Translation experiments with OBDV suggest that a large 224-240 kDa protein is produced, which ORFs 0-2 encode proteins involved in genome replication and silencing suppression, and ORFs 3-5 encode proteins involved in particle assembly, systemic movement, and aphid transmission. A non-structural, p17 protein encoded by the ORF 4 was previously identified as a host-dependent movement protein of PLRV. The two structural proteins, capsid protein (CP, ORF 3) and p80 (ORF 5), were known to be indispensable for the PLRV transmission by aphids. In a phloem-limited virus, systemic movement is a prerequisite for a successful acquisition of the virus by its aphid vector from the vascular system, and recently p17 was suspected to be involved in aphid transmission of PLRV. Here, we have studied tissue localization of the p17 protein in Nicotiana benthamiana plants infected with wild-type PLRV and a series of p17 mutants. Tissue localization was determined using p17-specific antibodies in a tissue immuno-binding assay. In plants infected with PLRV, p17 was found exclusively in the phloem, co-localizing in the same vascular tissue stained with antibodies specific to the PLRV capsid protein. Implications of p17 tissue localization for its possible role in PLRV aphid transmission will be discussed.

New method for establishing a network of operational warning of Septoria leaf blotch disease in winter wheat
M. EL JARROUDI (1), F. Giraud (2), P. Delfosse (3), L. Kouadio (1), L. Hoffmann (3), H. Maraite (4), B. Tychon (1)
(1) Univ of Liege, Arlon, BELGIUM; (2) Staphyt/BIORIZON, MARTILLAC, FRANCE; (3) Centre de Recherches Public Gabriel Lippmann, Belvaux, LUXEMBOURG; (4) Earth & Life Institute, Université catholique de Louvain (UCL), Louvain-la-Neuve, BELGIUM
Phytopathology 101:S47

A mechanistic model, PROCULTURE, based on commonly available meteorological and crop growth data and assessing in real time the risk of progression of septoria leaf blotch disease on winter wheat has been developed in Belgium and the Grand-Duchy of Luxembourg (GDL) to limit fungicide use. However, the reliability of meteorological stations used for the warning system varies according to the distance to the fields. A weather analysis based on the Fourier transform highlights a difference in the intraday variation between two sites in the GDL (Everland and Reuland). The correlation between these two sites is very high for the hourly temperature (R = 0.96), and for the hourly relative humidity (RH) (R = 0.86), (P < 0.05). However, the intraday variation (<11 hours) highlights contrasts for a given meteorological parameter. Hence, the correlation between temperature or RH decreased respectively from 0.96 to 0.43 and from 0.86 to 0.30. The comparison between infection conditions given by PROCULTURE using the Fourier transform, shows: (i) a positive but weak correlation between temperature at Reuland and Everland (R = 0.64), (ii) a good correlation between RH for these two sites (R = 0.86), and (iii) a contrasted difference for rain (R = 0.27), (P < 0.05). This Fourier transform based method enables to take into account the RH and temperature variation related to topography levels in the warning system and to understand and explain the variation in disease expression between a plateau and a valley bottom or between North and South slopes.

Regional-based typology of the main fungal diseases affecting winter wheat in the Grand-Duchy of Luxembourg
M. EL JARROUDI (1), F. Giraud (2), P. Delfosse (3), L. Kouadio (1), L. Hoffmann (3), H. Maraite (4), B. Tychon (1)
(1) Univ of Liege, Arlon, BELGIUM; (2) Staphyt/BIORIZON, MARTILLAC, FRANCE; (3) Centre de Recherches Public Gabriel Lippmann, Belvaux, LUXEMBOURG; (4) Earth & Life Institute, Université catholique de Louvain (UCL), Louvain-la-Neuve, BELGIUM
Phytopathology 101:S47

Despite its small territory size, the Grand-Duchy of Luxembourg (GDL) has several microclimates that result in a variability of disease severity between the South (Gutland) and the North (Oesling). Septoria leaf blotch disease of wheat is an important disease in the GDL. Over 2003–2009, the severity was strongly related to topography levels in the warning system and to understand and explain the variation in disease expression between a plateau and a valley bottom or between North and South slopes.
is wheat powdery mildew. The 2003 and 2009 cropping seasons showed the highest disease severity with 15% and 40%, respectively, in the Oesling whereas less than 1% severity was registered in the Gutland. Fusarium head blight was also present in the eastern part of the Gutland showing the highest prevalence and severity in 2007 and 2008 (8.5% and 8.3% respectively). These prevalence and severity percentages were significantly higher compared to the Oesling (% prevalence % severity, p = 0.049 and p = 0.012, respectively, Tukey’s test).

Deciphering the putative role of AoMDV1 in Ochratoxin A biosynthesis in Aspergillus ochraceus

K. EL MOUNADI (1), A. M. Fakhoury (1)

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Phytopathology 101-S48

Ochratoxin A (OTA) is a mycotoxin produced by several species of Aspergillus and Penicillium including Aspergillus ochraceus, an important OTA producer in cereals. Since OTA is nephrotoxic, teratogenic and carcinogenic for both humans and animals, several countries have established action levels to regulate its presence in some agricultural products. The AoMDV1 gene, encoding a protein involved in mitochondrial division, has been previously disrupted in Aspergillus ochraceus and the resulting AoMDV1 transformant was found not able to produce OTA. In an attempt to understand the involvement of AoMDV1 in OTA biosynthesis and identify additional genes regulating the biosynthesis of OTA in A. ochraceus, a cDNA-AFLP differential display screening was performed with the AoMDV1 transformant (MDV9) (OTA-) and a wild type strain of A. ochraceus (OTA+) NRL 5175. Twenty five transcript-derived fragments (TDFs) were up-regulated in the OTA+ strain compared to the AoMDV1 strain. They included TDFs with high sequence homology to genes involved in the regulation of signal transduction, the biogenesis and metabolism of mitochondria and peroxisomes and in mediating stress response. A Yeast two hybrid screen was also used to identify interacting partners of AoMDV1 under conditions conducive to the biosynthesis of OTA.

Volunteer stream monitoring for invasive Phytophthora species in western Washington

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Phytopathology 101-S48

To supplement state agencies in their monitoring for Phytophthora ramorum, the sudden oak death (SOD) pathogen, a community-based stream monitoring program was initiated in 2010. This project expands on the streams currently being sampled by the WA Dept. of Natural Resources (WADNR) as part of the national P. ramorum survey and on nursery surveys by WA State Department of Agriculture (WSDA) to allow for early detection of P. ramorum and other invasive Phytophthora species, as well as examining the biodiversity of Phytophthora spp. in stream ecosystems. This project provides an opportunity to increase public awareness of waterborne plant pathogens and the damage they cause. The baiting process involved placing rhodendron leaves in mesh bags and deploying them in streams for two weeks. After bait retrieval the leaves were cultured on selective media and colonies of Phytophthora were isolated on V8 agar. Phytophthora species were identified using molecular and cultural methods. Several species of Phytophthora and Pythium were identified from stream samples and no P. ramorum was found in 2010. Volunteers included Master Gardeners, high school, community college, university students, and other individuals. Some students worked on group projects related to Phytophthora in the lab at WSU-Puyallup. The program was expanded in 2011 with more baiting sites and student involvement.

APS has many resources available for members. If you are hosting a career fair, trying to recruit undergrads, interacting with your local community, teaching, writing, or speaking about Plant Pathology, APS can help. APSnet offers electronic information instantly, as does the APS channel on YouTube, and the APS community of Facebook. Members can take advantage of traveling displays, career brochures, and News Features. The Office of Public Relations and Outreach (OPRO) will showcase services members can appreciate.

Mapping partial resistance to Pythium irregulare in the soybean accession PI 424354

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Phytopathology 101-S48

Pythium irregulare causes damping-off of soybean and has emerged as an important soybean seedling pathogen in Ohio. The objective of this research was to identify major quantitative trait loci (QTLs) in the plant introduction PI 424354 that confers resistance to P. irregulare. Two BC2F3 populations were used in this study including: 224 recombinant inbred lines (RILs) of OHS 303 (partially susceptible) × (Williams (susceptible) × PI 424354) and 128 RILs of Dennison (susceptible) × (Williams x PI 424354). Seeds from each RIL were planted into a colonized sand-cornmeal mixture. Data was collected from 2-week old seedlings for percent germination, total weight (g), root weight (g), and a root rot score using an ordinal scale. Based on the analysis of the phenotype data for both populations, there was a significant difference between lines (P < 0.0001) for root weight and root rot scores, and the data for both populations fit the model for quantitative resistance. A combination of SNP data from the BeadXpress and SSR will be used to construct the genetic map and identify the QTLs. These results suggest that this soybean accession can be an important source of partial resistance in developing germplasm for breeding new cultivars with more durable resistance to P. irregulare.

Mapping partial resistance to Fusarium graminearum in ‘Conrad’ soybean

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Phytopathology 101-S48

Fusarium graminearum has emerged as an important soybean seedling pathogen in Ohio. The objective of this research was to identify the major quantitative trait loci (QTLs) conferring resistance to F. graminearum in a Conrad (Resistant) x Sloan (Susceptible) F2 population. A rolled-towel assay was used to phenotype 262 recombinant inbred lines (RILs). Twenty seeds from each RIL were placed on a germination towel and inoculated with 2.5 × 106 macroconidia/ml, the towels were rolled and placed in a bucket in the dark. An experimental design was a randomized block design, in which replicates and replications of the RILs were set up over time. At seven days after inoculation, the lesion and plant lengths were measured for each seedling, and the proportion of the seedling affected (lesion length/ plant length) was calculated as a measure of disease severity. The mean disease severity for each RIL was then analyzed using best linear unbiased predictor. Based on the analysis, the phenotype data was divided into a high and a low model for quantitative resistance. A total of 208 SSR and SNP markers were screened and a map of 172 markers was developed using JoinMap, and QTLs were identified using MapQTL. Conrad is a major source of partial resistance to Phytophthora sojae, this comparison of two root pathogens for overlapping QTLs associated with resistance will provide key clues to both basal resistance and pathogen specific resistance in this cultivar.

Partial saturation of potted ornamentals reduces Pythium root rot on flooded floor greenhouses

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Phytopathology 101-S48

Sub-irrigation of potted ornamentals has become a useful means for growers to manage and reduce Pythium root rot. However, a major concern for growers has been the potential for spread of disease organisms namely Pythium spp. We observed that current ebb&flow watering systems operate slowly and allow the root medium to approach saturation during each watering cycle. Growers have no ability to restrict the amount of water provided to the plants. We designed a flooded floor system to rapidly deliver and drain water, thus providing a partial saturation (PS) of the rooting media. This model caused less root rot as compared to flooded floor greenhouses.
mums, and geraniums were equally inoculated with virulent Pythium spp., placed on flooded floors along other plants and exposed to a conventional saturation (CS), the percentage of root rot was 63, 58, and 25%, respectively. When these crops were similarly inoculated and exposed to PS that produced an average 10% less volumetric water content, the amount of root rot was reduced to 41, 43, and 5%, respectively. The water stress resulting from PS treatment reduced biomass and stem height by 10 to 20% compared to CS. In general, crop quality was improved by PS, because plants were more compact, so they could be held at production spacing for a longer time before quality declined.

University of Arkansas Soybean Disease Screening Project

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Phytopathology 101:S49

Since 1990, Arkansas has maintained one of the most comprehensive soybean disease screening programs in the southern U.S., thanks to the Arkansas Soybean Promotion Board. A combination of inoculated field nurseries and greenhouse test are used to screen all varieties entered into the official University of Arkansas Variety Testing Program (OVT) each year to obtain disease ratings to all the major diseases in Arkansas. Our disease ratings are reported in the Arkansas Variety Testing Report, Arkansas Soybean Update, and the SOYVA variety selection program. Currently, the University of Arkansas has developed field nurseries at the Southwest Research and Extension Center and at the Newport Research Station for evaluating frug-eye leaf spot, aerial web blight, and stem canker. Supplemental inoculum of a specific pathogen is applied throughout the growing season to help ensure adequate disease pressure for evaluations. Plots are visually rated at the R5 growth stage using a 0 – 9 scale. Soybean cyst (races 2, 3, 5, 9 and 14), root knot nematode, and reniform nematode resistance screenings are conducted in greenhouses at the Southwest Research and Extension Center and the Crowley Warren laboratory on the University of Arkansas Fayetteville Campus. Diseases cost $250,000,000 per year in lost yield and quality statewide, by some estimates. This program has provided producers comprehensive information on the resistance package for each variety, lowering the risk and economical loss due diseases and nematodes.

Seasonal synchrony between pheromone trap catches of the bean bug, Riptortus pedestris and the timing of invasion into soybean fields

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Phytopathology 101:S49

Seasonal catches of the synthetic pheromone of the bean bug, Riptortus pedestris (Heteroptera: Alydidae), captured in traps containing the synthetic pheromone were investigated under different field conditions from 2005 to 2007. In soybean fields, the number of bugs attracted to the pheromone traps increased after flowering and peaked at 9 – 13 days after flowering. After these attractive emissions of pheromone were observed was YMMV and yam-infecting badnaviruses. These results indicate that yam-infecting badnaviruses are an emerging viral threat in the yam system in West Africa and highlight the urgent need to produce yam varieties with multiple virus resistance particularly with resistance to yam-infecting badnaviruses.

Occurrence of citrus quick decline in California

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Phytopathology 101:S49

In the summer of 2009 trees were rapidly declining and dying within a few weeks on an estimated 150–200 acres of orchard in Tulare County, at the San Joaquin Valley of California. Bad union symptoms included “honeycomb” or “inverse stem pitting” on the side of the bark. These symptoms were observed on sweet orange, grapefruit, and tangerines on Sour Orange (SO) rootstock. Additionally, roots showed grayish brown to purple lesions in the bark of large scaffold roots, which is characteristic of dry root rot symptoms caused by Fusarium solani. Leaf, shoot, and bark material were tested for Citrus tristeza virus (CTV) using Enzyme-Linked Immunosorbent Assay (ELISA), and Reverse Transcription Polymerase Chain Reaction (RT-PCR). Roots were plated onto different culture media in order to isolate fungal and bacterial pathogens. Fungal and bacterial cultures were further processed for molecular identification. To assess the girdling at the bud union by CTV, presence of starch in roots was determined by dipping roots in 2% potassium iodide and 0.2% elemental iodine solution. Molecular and ELISA analyses of plant samples showed that declining trees were consistently infected with P. citri and CTV were recovered from healthy looking trees. The roots of declining trees were also starch depleted, indicating plants stressed CTV induced girdling at the bud union. We hypothesize that interactions between CTV and F. solani potentially play role in the quick decline problem in Tulare County.

Identification of different species causing Botryosphaeriaceae canker in citrus reveal Neofusicoccum mangiferae with Stylatulidium-like synanomorph

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Phytopathology 101:S49

Branch/trunk cankers of citrus caused by members of the Botryosphaeriaceae can sometimes lead to serious decline or death of branches and whole plants. A study was conducted to determine the different species causing the disease on citrus and their geographical distribution in California. Orchards in six citrus growing California counties- Fresno, Riverside, San Diego, San Luis Obispo (SLO), Tulare, and Ventura - were studied. Infected samples were collected from which isolates were obtained and identified using morphology and molecular methods with three markers - Internal Transcribed Spacer [ITS], Beta Tubulin, and Translation Elongation Factor. Nine different species (Dothiorella viticola, D. iberica, Neofusicoccum mangiferae, N. mediterraneun, N. luteum, N. australe, N. parvum, N. luteum, and Diplodia mitula) have been identified and tested to be causing citrus canker. All ages of trees and commonly used rootstocks - Carizzo, Volkameriana, and Sour
Orange - were infected. During morphological identification of \textit{N. mangiferae}, multiple conidia types were found including septate conidia with a dark central region as well as \textit{Scytalidium}-like synanamorph. These conidia types were initially described by Sutton and Dyko (1989). Although isolates of the organism have been studied in the Nethelands, South America, Australia, etc., the conidia types have not been found elsewhere.

**First report of \textit{Raffaelea canadensis} showing laurel wilt disease symptoms on avocado in California**

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Phytopathology 101:S50

Laurel wilt disease caused by the fungus \textit{Raffaelea lauricola} and vectored by a non-native redbay ambrosia beetle, was first detected in Georgia in 2003. Laurel wilt has caused extensive mortality of native redbay in Georgia, Florida, South Carolina, and recently Mississippi. During a survey in 2010 in Temecula, CA, avocado orchard with a history of root rot, an avocado (cv. Hass) tree, was found showing typical laurel wilt disease symptoms. The crown was declining, and it exhibited dead branches without leaves. Black-to-brown discolored sapwood under the bark and many ambrosia beetle exit holes within 1 to 1.5 in up the bole were also observed. A \textit{Raffaelea sp.} was consistently isolated from symptomatic tissues plated onto cycloheximide-streptomycin malt agar and incubated at room temperature for two weeks. Small subunit (18S) sequences of rDNA were amplified using primers NS1 and NS4. A BLASTn search of all sequences revealed high homology to \textit{Raffaelea canadensis}. Pathogenicity testing was conducted by pipetting 50 µl of a 10^3 conidia per ml suspension using two isolates into 2 mm diameter holes drilled into avocado fruit (cv. Hass) trees (10–15 cm DBH). Sterile water was used as a control in five 2 mm diameter holes on each tree. \textit{R. canadensis} was consistently re-isolated from necrotic tissue but not from control treatments. To our knowledge, this is the first report of \textit{R. canadensis} associated with ambrosia beetle (\textit{Xyleborus sp.}) causing wilt on avocado in California.

**Perception by growers and consultants of the importance of corn diseases**

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Phytopathology 101:S50

To improve our knowledge about perceptions of corn disease management for successful corn production, a multi-state survey was conducted in Illinois, Iowa, Ohio, and Wisconsin in 2010. An equal number of surveys were sent out to randomly selected growers and consultants (n = 188 for each group and state for total of 1,504 surveys). The valid response rate was 47%. Preliminary data analyses have been conducted. In both grower and consultant state for a total of 1,504 surveys). The valid response rate was 47%. Preliminary findings indicated that consultants typically spend 30–60% of their time on disease management, while growers spend 5th to 7th of their time on disease management. Among the diseases considered more important for managing than insects or diseases. Among different disease categories, stalk rots were considered a significant risk to annual corn production, although there was some variation in responses across states and groups. However, most consultants and growers considered themselves only good (mid-rating) at disease diagnostics. These results suggest that further efforts are needed to improve knowledge of the impact of corn diseases on an annual basis.

**Does one size fit all for delivering corn disease-related information?**

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Phytopathology 101:S50

With advanced technologies like Twitter, blogs, and Facebook, are there better ways we can package educational material to meet the needs of our clientele? To improve our knowledge about the use of information and technology, a multi-state survey was conducted in Illinois, Iowa, Ohio, and Wisconsin with corn growers and consultants in 2010. An equal number of surveys were sent out to randomly selected growers and consultants (n = 188 for each group and state for total of 1,504 surveys). The valid response rate was 47%. Preliminary findings indicated that consultants typically spend 30–45 minutes per meeting with their grower clients. Communications using telephone or email were <6 times and <3 times per month, respectively. Consultants are more likely to use the internet to gather information, although internet use is >50% for both groups. Consultants also are more likely to bookmark ag-related materials. For consultants, email is a valuable tool and they typically sign up to email lists from University extension and industry. Both University extension and seed dealers were considered valuable sources of information. The use of newspapers, radio, and TV only were considered slightly important. When considering the amount of time spent with clients by consultants, there is great potential for providing information to key clientele more effectively through the use of advanced technologies.

**Design and validation of queries for the detection of \textit{Phytophthora ramorum} in simulated metagenomes**

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Phytopathology 101:S50

The main goal of this investigation was to validate a queries design method for the detection of Straminopile plant pathogens using massively parallel sequencing as a diagnostic tool. Unique pathogen diagnostic queries were obtained from the genome of \textit{Phytophthora ramorum}. A PERL script, based on a modified Tools for Fingerprint Identification (TOFI) script, was used to obtain \textit{P. ramorum} diagnostic queries of 20, 40, 60, 80, 100, 120, and 140 bp. Mock Sample Databases (MSD) containing the pathogen and the host genomes mixed at various ratios were simulated using MetaSim, a sequencing simulation program. Finally, a BLAST of all queries against the MSD was performed, and numbers of pathogen query hits and matches assessed. The number of hits and matches increased as the length of queries decreased, with the exception of 20 bp queries that generated less matches than 40 bp queries. At pathogen abundance values of more than 0.5%, the percentage of positive hits was beyond 95% of total hits. Queries 40 bp lengths blasted against MSDs with pathogen abundance between 0.01% and 0.5% needed 580 reads to reach 10 matches, suggesting good potential for high-throughput diagnostics.

**Design and validation of queries for the detection of \textit{Puccinia graminis} in simulated metagenomes**

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Phytopathology 101:S50

The main goal of this investigation was to validate a queries design method for the detection of fungal plant pathogens using massively parallel sequencing as a diagnostic tool. Unique pathogen diagnostic queries were obtained from the genome of \textit{Puccinia graminis}. A PERL script, based on a modified Tools for Fingerprint Identification (TOFI) script, was used to obtain \textit{P. graminis} diagnostic queries of 20, 40, 60, 80, 100, 120, and 140 bp. Mock Sample Databases (MSD) containing the pathogen and the host genomes mixed at various ratios were simulated using MetaSim, a sequencing simulation program. After blasting all queries against MSDs with variable pathogen concentrations, hits and matches were assessed. The numbers of queries obtained for \textit{P. graminis} were: 594209 (20 bp), 175895 (40 bp), 59986 (60 bp), 21790 (80 bp), 8108 (100 bp), 3131 (120 bp), and 1294 (140 bp). Queries with 20 bp length showed the highest number of matches at all pathogen abundance values under 25%. At pathogen abundance values of more than 0.5%, the percentage of positive hits was beyond 87% of total hits. The number of hits and matches increased as the length of queries decreased. The number of reads needed to reach 10 matches increased as pathogen abundance decreased. Queries 20 bp lengths blasted against a MSD with pathogen abundance between 0.5% and 0.01% needed 85 reads to reach 10 matches. Queries 20 bp long showed the most potential for diagnostics, reaching 98.28% of positive hits.

**Chemical management of Fusarium wilt of watermelon in the eastern U.S.A.**

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Phytopathology 101:S50

Yield loss due to Fusarium wilt is a re-emerging problem in watermelon in the U.S. because of the increasing production of triploid cultivars, which lack host
resistance, and the emergence of the virulent race 2 of Fusarium oxysporum f. sp. niveum. One potential management strategy is the use of soil applied fungicides to reduce Fusarium wilt. The U.S. national program, inter-regional project 4 (IR-4), initiated trials of soil applied chemicals to manage Fusarium wilt of watermelon in 2007. Subsequent field trials in Maryland, Indiana and Delaware in 2008 and 2009 indicated that the fungicides acibenzolar-S-methyl and thiophanate-methyl may be effective soil-applied fungicides. Prothioconazole alone or in combination with other fungicides reduced Fusarium wilt in 2009 in Maryland. In Indiana, however, prothioconazole reduced wilt only when used alone or with thiophanate-methyl. One additional trial was conducted in 2010 in Maryland. Acibenzolar-S-methyl alone or in combination with prothioconazole and thiophanate-methyl reduced wilt in that location. While acibenzolar-S-methyl, prothioconazole and thiophanate-methyl fungicides have shown promise in reducing wilt in some environments, the variation in efficacy across tests and over environments indicates that further evaluation is necessary.

First report of “Candidatus Phytoplasma asteris” (Group 16Sr1) infecting fruits and vegetables in Islamabad, Pakistan

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Phytopathology 101:S51

Nearby fruit and vegetable fields in Islamabad, Pakistan were surveyed for phytoplasma infection. “Candidatus Phytoplasma asteris” (Group 16Sr1) was found infecting mango, citrus, loquat, geranium, periwinkle, radish, blackberry and potato. Results suggest that a polyphagous vector may be involved in phytoplasma transmission to these plant species, which are first host records of 16Sr1 phytoplasma infection in Pakistan.

Morphological, pathological, and molecular characterization of lupin anthracnose and its relationship with tamarillo anthracnose in Ecuadorian Andes

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Phytopathology 101:S51

Anthracnose, caused by Colletotrichum acutatum, is a serious problem of lupin (Lupinus mutabilis) in Ecuador and worldwide, tamarillo (Solanum betaceae) and many other hosts. Morphological features, host specificity test, specie-specific and ITS-PCR sequence analyses were used to characterize Colletotrichum isolates from lupin and tamarillo. All Colletotrichum isolates from lupin and tamarillo tested positive with C. acutatum-specific polymerase chain reaction (PCR). Colony diameter, spore shape, and insensitivity to benomyl grouped the lupin and tamarillo anthracnose isolates closer to C. acutatum. Host preference test demonstrated a positive cross-reaction among C. acutatum from lupin and tamarillo. Isolates were more virulent when they were inoculated on their own host. The phylogenetic relation among isolates of lupin and tamarillo was compared with other Colletotrichum isolates from hosts around the world. Comparative analysis with a range of reference ITS sequences identified the isolates from lupin and tamarillo anthracnose as C. acutatum. Analysis of internal transcribed spacer (ITS) sequences of Colletotrichum isolates clustered lupin and tamarillo isolates from Ecuadorian Andean zone into two separate subgroups. Molecular analyses indicated that the C. acutatum from Andean lupin is distinct from other C. acutatum lupin populations around the world. Project financially supported by TELFUN project /WUR and ESPE University, Ecuador. Recognition to Marco Rivera, Pablo Landazuri and Cynthia Rosas.

Detection of tospoviruses infecting Hymenocallis littoraris and Hippeastrum vittatum in Kunning, China

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Phytopathology 101:S51

The leaf samples of Hymenocallis littoraris (Jacq.) Salisb. and Hippeastrum vittatum (L., Her) Herb., showing chlorotic spot symptoms, were collected from Kunning, capital of Yunnan province, China. In order to confirm the infection of tospovirus, the samples were tested using both electromagnetic and ELISA. The quasi-spherical particle 80–85 nm in diameter were observed in the leaf diseased leaves of both H. littoraris and H. vittatum through negative staining method. Tospovirus-like particles were found to exist single in line in metaplasm in H. littoraris and multiply in mass in the vesicle of H. vittatum. The results confirmed that H. littoraris and H. vittatum were infected by tospoviruses.

Population genetic analysis of Leptographium longiclavatum and a pathogen associate with the mountain pine beetle Dendroctonus ponderosae

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Phytopathology 101:S51

The mountain pine beetle (MPB) and its fungal symbiont (Leptographium longiclavatum) have destroyed over 16 million ha of pine forests in Canada, the largest epidemic in recorded history. The fungal symbionts could play an important role in the epidemics by reducing the tree defense response following the beetle colonization. We investigated the genetic structure of L. longiclavatum isolated from various populations in Western North America using microsatellite markers. Based on Bayesian clustering inference, we found that there are two clusters that are concordant with geographic origin. One cluster comprises individuals from Northern sites where the beetle-fungus complex has been established and a second cluster is found along the Rocky Mountains. This distribution pattern is best explained by geographic origin and is concordant with the patterns observed in the beetle and with Grossmannia clavigera, another important, pathogenic fungal symbiont of the MPB. The general agreement in north-south differentiation of L. longiclavatum and G. clavigera populations, as well as the MPB suggests the dependence of fungal dispersal on their bark beetle vector and similar demographic processes in these two fungi. This information is important for disease management and surveillance.

A new broad-spectrum fungicide for use in row crops

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Phytopathology 101:S51

Priaxor™ is a new broad-spectrum premix fungicide under development in the United States by BASF Corporation containing a 2:1 ratio of the active ingredients pyraclostrobin and fluxapyroxad. Priaxor has been submitted to the EPA for potential registration on several major row crops including soybean, corn, wheat, barley, and canola. Research has indicated Priaxor is highly effective at controlling several important diseases of row crops including gray leaf spot (Cercospora zeae-maydis) and northern corn leaf spot (Exserohilum turcicum) in corn as well as brown spot (Septoria glycinum) and frogeye leaf spot (Cercospora sojina) in soybean. Priaxor has demonstrated excellent disease control of the Septoria diseases of wheat (Septoria tritici and S. nodorum) in research trials. In barley, high levels of control have been achieved on net blotch (Pyrenophora teres) and scald (Rhynchosporium secalis). The combination of two active ingredients with different modes of action will reduce the risk of fungicide resistance development in the target pathogens. Trial results from 2009 and 2010 will be presented. EPA registration is expected in 2012.

Early activation of defense genes in kumquat by the citrus canker pathogen

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Phytopathology 101:S51

Citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), is an economically important disease that affects various citrus types around the world. During previous work we showed that kumquat [Fortunella margarita (Lour.) Swingle] was resistant to citrus canker, the result of an active defense response. In this study we compared the induction in kumquat and susceptible grapefruit (Citrus paradisi Macf.) of a number of defense genes associated with PAMP-triggered immunity (PTI, or basal defense) and effector triggered immunity (ETI, or R-mediated resistance), the latter often leading to systemic acquired resistance (SAR). Our results showed that, generally, defense genes were induced earlier and to higher levels in the resistant kumquat after inoculation with Xcc. In addition, three genes belonging to the NPR1 family were differentially expressed between the two citrus types. This is important because in other species these genes are central in the induction of SAR and function either as positive or negative regulators of a number of downstream genes. We also compared the response of the two citrus species when the peptide Flagellin22 from Xcc (a PAMP) was used instead of the pathogen. The response to this treatment was less intense than the response observed after Xcc inoculation. Our results indicate that an earlier and enhanced activation of defense genes may play an important role in the defense mechanism of resistance to citrus canker observed in kumquat.

Efficacy of OMRI-certified fungicides and chitosan to manage early blight and septoria leaf spot in tomato

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Phytopathology 101:S51
Field studies to evaluate the efficacy of OMRI-certified and other materials for control of early blight and septoria leaf spot of tomato were conducted in Lexington, Kentucky in 2009 and 2010. Nine fungicides as well as ammonium bicarbonate and chitosan were evaluated in an organic production system. The most effective fungicides to manage septoria leaf spot and early blight of tomato were copper-based fungicides. None of the biological-based products (Sonata® and Serenade Max®), plant-based extracts (Trilogy® and Regalia® SC), chitosan, ammonium bicarbonate nor horticultural lime sulfur provided a significant (P > 0.05) reduction in disease severity However, in spite of significant (P < 0.05) disease control in plots treated with copper-based products, no significant (P > 0.05) improvement in yield over the untreated control was observed during the first two experiments, in which the initial symptoms of foliar disease were observed after fruits were set. In the third field trial, in which initial symptoms were observed before fruit set, Serenade Max®, Bordeaux mixture, Regalia® SC, water-soluble chitosan and lime sulfur improved yield, although none provided significant disease control.

Characterization of new races (races 11 and 12) and several novel strains of spinach downy mildew pathogen Peronospora farinosa f. sp. spinaeae C. FENG (1), J. C. Correll (1), K. E. Kammeijer (2), S. T. Koike (2) (1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) University of California Cooperative Extension, Salinas, CA, U.S.A.
Phytopathology 101:S52

Spinach downy mildew disease, caused by the obligate pathogen Peronospora farinosa f. sp. spinaeae (Pfs), is the most destructive disease for spinach production. New races of this pathogen have been emerging at a rapid pace during the last two decades as a result of the high-density, year-around production. Many new races of this pathogen have been identified in different areas such as the result of the high-density, year-around spinach production in California. Up to 2007, 10 races of Pfs had been identified and the spinach resistance locus RPF2 provides resistance to races 1-10. Race 11 was identified in 2008 and could overcome the resistance of race 1-10 resistant cultivars. Spinach resistance loci RPF1, RPF3 and RPF6 can provide resistance to this race. Race 12, recently sanctioned by the International Working Group on Peronospora (IWGP), was identified in 2009 that could overcome the resistances of RPF1 and RPF2. The RPF3 locus was effective for race 12. In 2010, a novel deviating strain, UA0510C, was found to be virulent to RPF2 and RPF3 containing cultivars, and only the RPF1 locus was effective to this isolate. Another novel deviating isolate, UA4410, and race 12 caused identical disease responses on differentials, but UA4410 could be distinguished from race 12 by its ability to infect a number of additional cultivars including Pigeon, Zebu, Finch, and Celesta. A total of 150 spinach cultivars and breeding lines have been evaluated for resistance to races 10, 11, and 12 as well as deviating isolates UA510C and UA4410.

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The induction of at least eight caspase-like activities has been described under different stress conditions and during PCD in plants. The aim of this work was to determine if caspase-like proteolytic activity is involved in P. infestans (Pi) induced PCD in Solanum tuberosum leaves. Protein extracts from Pi infected leaves were prepared and proteolytic DEVDAse activity was measured at different times after infection. Results obtained show that DEVDAse activity was increased in a 70 and 80% at 24 and 48 h after Pi infection, respectively. In order to characterize the activity detected, the effect of caspase and general inhibitors was analyzed. Only Ac-DEVD-CHO (50 µM), a specific caspase-3 inhibitor and PMSF, a serine protease inhibitor (2.5 mM) were able to reduce the activity in a 95 and 50% respectively. Additionally, the effect of several ions on the potato-caspase-3 like activity was determined. DEVDAse activity was sensitive to CaCl2 (500 mM) lossing 40% of activity and CuCl2 (50 and 100 mM) which enhanced it 6 folds. All concentrations assayed of NaCl (0 to 500 mM) had no effect. It is known that animal caspase-3 and plant DEVDAse activities are active at neutral pH. In contrast, this caspase-3 like activity is active at acidic pH, suggesting activation after cytoplasm acidification or a vacuolar/intermembrane mitochondrial localization. This work constitutes the first evidence and characterization of caspase-like activity during S. tuberosum-Pi interaction.

Transcriptome analysis of a wheat cultivar infected by different chemotypes of Fusarium graminearum D. G. FERNANDO (1), K. Al-Taweel (1), A. Brule-Babel (1) (1) University of Manitoba, Winnipeg, MB, CANADA
Phytopathology 101:S52

Fusarium head blight (FHB), caused by species of the fungus Fusarium, is a worldwide disease of wheat (Triticum aestivum L.). FHB disease can cause animal feed refusal/sickness and illness in humans by producing mycotoxins that consist of nivalenol (NIV), deoxynivalenol (DON), 3 deoxynivalenol (3DON), and 15 deoxynivalenol (15DON). F. graminearum isolates with a 3DON chemotype are displacing the predominant 15DON isolates in many parts of North America. 3DON isolates have been found to produce significantly higher levels of DON than those with a 15DON chemotype. To get a clear insight to the host genes involved in defense response when Fusarium infected plants and to analyze the expression profiles of these genes in both; 3 DON-infected plants and 15 DON-infected plants, suppressive subtractive hybridization (SSH) method and quantitative real-time PCR (qRT-PCR) were carried out. Twenty up and down-regulated genes were identified. Of the genes that had matches to known genes present in the NCBI database, several had roles related to plant defense and stress tolerance. Five putative defense-related genes were confirmed by qRT-PCR. Several-fold higher induction of the putative genes in the 3 DON-infected genotypes “Sumai3” compared with a control, indicates a putative role in the resistance response to Fusarium graminearum. Additionally, the expression profile of the two infected plants (by 3DON & 15 DON) varied between sampling times post inoculation.

Field efficacy of novel fungicides for the control of Sclerotium cepivorum in California A. E. FERRY (1), M. Davis (1) (1) University of California, Davis, Davis, CA, U.S.A.
Phytopathology 101:S52

White rot of alliums, caused by the pathogen Sclerotium cepivorum, is a devastating disease of onion and garlic worldwide. A combination of sclerotia stimulants and fungicides is currently recommended to control the disease. A field study was conducted in Tulelake, California, to determine the efficacy of five different fungicides for the control of white rot on onion, including two novel fungicides: penthiopyrad (LEM17) and fluopyram (Luna Privilege). The other fungicides investigated were tebuconazole (Folicur), boscalid (Endura), and fludioxonil (Switch). Disease was measured as incidence of kilograms of diseased and healthy bulbs at harvest. Means separation was determined using the Tukey test. Tebuconazole and penthiopyrad were most effective; both treatments reduced white rot incidence by over 50%. Fluopyram was moderately effective, and reduced incidence by 30%. Fludioxonil and boscalid did not significantly influence disease severity. No phytotoxicity was observed in any of the treatments. These data provide evidence of potential new chemical treatments for the control of S. cepivorum.

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Geotrichum candidum is the causal agent of sour rot in tomato and other fresh produce. This disease is a limiting factor of tomato production on the Eastern Shore of Virginia and can cause major losses during post-harvest handling. Infections by G. candidum are most prevalent during periods of wet harvest conditions or abrupt temperature changes and if improper post-harvest handling procedures are employed. There are currently no in-field treatments targeted to prevent sour rot infections so post-harvest treatments are used to prevent further losses. Currently, the methodology used involves placing newly harvested fruits and vegetables in a conducive environment and involves severe wounding to inoculate with G. candidum. This practice does not accurately reflect natural fruit infections and response to post-harvest treatments may differ under these artificial methods. This compelled us to develop a method of infection without wounding. Bartz (2000) showed that tomatoes cooled with Rhizopus water suspensions developed infection, though the majority of studies focus on bacterial cells entering tomatoes and there is a lack of information on internalizing fungal pathogens. As a result, a vacuum pressure method of internalizing G. candidum spores was developed to internalize spores into tomato fruit to accurately imitate infected fruit for further management studies.

Influence of weather factors on panicle blast in upland rice in Brazil M. C. FILIPPI (1), V. L. Silva-Lobo (1), G. B. Silva (2), A. S. Prabhu (1), R. S. Figueiredo (3) (1) EMBRAPA-CNPAF, Santo Antonio De Goiás, BRAZIL; (2) UFRA, Belem, BRAZIL; (3) UFG, Goiânia, BRAZIL
Phytopathology 101:S52

Panicle blast (Magnaporthe oryzae) is responsible for severe grain yield losses in upland rice. A field experiment was conducted with rice cultivar BRS Bonança to identify the meteorological factors that determine the panicle blast severity. The layout was a split plot design with three replications. The treatments consisted of 12 weekly plantings and five levels of nitrogen. Twenty panicles per plot which emerged at the same time (50% flowering),
Synergy in biorational insecticides used on collard greens, *Brassica oleracea*, infested with diamondback moth, *Platella xylostella*  
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Phytopathology 101:S53

Climate change will have a progressively negative effect on crop yields with further reductions arising through increased severity of plant disease, particularly vector-borne disease. While some pathogens' life cycles will be limited by increasing temperatures, other climatic, such as elevated (e) CO₂, may provide more favourable conditions for some pathogens. Field based experiments from the Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE) experiment and closed environment chambers investigated the effects of elevated (e) CO₂ on wheat pathogens such as wheat stripe rust (*Puccinia striiformis*), crown rot (*Fusarium pseudograminearum*) and Cereal Yellow Dwarf Virus vectored by the aphid Rhopalosiphum padi. *P. striiformis* disease progress and fecundity was not affected by eCO₂ but high temperatures during the growing season may limit development and survival of the disease in some regions. Conversely, eCO₂ will increase *F. pseudograminearum* biomass while saprophytic fitness remains stable, potentially leading to rapid colonization of stubble harvest and an increased incidence of crown rot in future climates. Preliminary investigations of *R. padi* grown in eCO₂ indicated no significant differences in development but reductions in fecundity for nymphs. Using an electrical penetration graph (EPG) to study feeding behaviour it was found *R. padi* probing less but spend more time ingesting from wheat grown under eCO₂ potentially leading to a lower incidence of virus transmission.

**Interaction among powdery mildew (*Blumeria graminis*) and triticate (*x Triticosecale*) in Germany as a model for pathosystem analysis**  
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Phytopathology 101:S53

Triticale, the intergeneric cross between wheat and rye, was highly resistant to powdery mildew before 2004, but has revealed an increasing susceptibility since then. Breeding of cultivars with durable resistance requires precise knowledge about virulence structure of pathogen populations and genetics of host resistance. The German triticale mildew population was monitored by analysing 694 single-pustule isolates in 2007–2010 with a new differential set of 272 pathotypes and high Simpson indices (0.95–0.97) within years. Most of the isolates (80%) were highly complex with at least 14 virulences. In detached leaf segment tests, only 16 out of 826 (2%) breeding lines and the triticale cultivar Grenado exhibited complete seedling resistance. The same genotypes showed effective adult-plant resistance at six field locations in three years. Analysis of primary triticate produced by crossing durum and aesimum wheat with clothing and race-specific resistance genes and rye inbred lines showed that the outcome is not predictable and expression of resistance genes is strongly affected by the rye genome. By genetic mapping of race-specific resistance genes, a dominant monogenic inheritance has been identified in each of six tritice line. In conclusion, the use of race-specific resistances seems to have a restricted durability only.

**Could viruses of wheat prevent supply meeting demand?**  
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Phytopathology 101:S53

Global wheat production must increase by 50% by 2050 to meet projected requirements. While Defra statistics show that the yield of wheat in the UK increased from 1940 until 2000, it then began to plateau. It has been suggested that this could be due to virus infections suppressing yield. Exploring this possibility is important, and begins with high throughput diagnostics to assess the prevalence of viruses in UK wheat. Real-time PCR is well suited to this, and assays have been developed for a selection of viruses that have been found in wheat. and other related plants in the UK. Thus far approximately 600 samples of wheat from across the UK have been screened for six of these viruses. Just five samples were positive. Assays are being developed for the remaining viruses to complete this work. Since there is such a low prevalence of these known viruses it has been hypothesized that the problems are due to as yet unidentified viruses. Therefore the project will move in an exciting new direction by exploiting next generation sequencing methods to investigate the complete virome present in wheat and its surrounding environment (weeds, hedgerow plants and possible vector samples). In comparison to specific assays, which are biased and only include viruses that are currently identified and characterised, a comprehensive picture of the hidden diversity in each sample will be produced; this information may hold the key to current and future threats to wheat production.

**Comparison of the ergot alkaloid synthesis (EAS) gene cluster among Clavicipitaceae fungi**  
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Phytopathology 101:S53

Genome sequence analyses of several fungi belonging to family Clavicipitaceae allowed comparison of ergot alkaloid synthesis (EAS) gene clusters. These fungi exhibit diverse associations with their host plants, from symbiotic mutualism with grasses (e.g., *Epichloë* *Neotyphodium* spp. in Poaceae) and morning glories (*Periglandula* spp. in Convolvulaceae), to ergot (*Claviceps* spp.) infection of cereals. Animal toxicosis due to ergot alkaloids (EA) in *Neotyphodium coenophialum*-infected tall fescue have a major impact on U.S. livestock production, with annual losses close to $1 billion. Characterization of the *Claviceps* gene cluster identified the presence of eight core genes required for the production of the simplest EA (clavines and lysergic acid), and additionalanking genes encoding nonribosomal peptide synthetases (*ipsA, ipsB* and *ipsC*) are required for the more complex ergopeptines with enhanced pharmacological activities. The presence, order and orientation of the EAS genes as well as the length of the clusters were compared, and in most Clavicipitaceae the gene cluster arrangement was similar to the *C. purpurea*, and a novel gene (*easP*) has been identified in EAS clusters of *P. ipomoeae* and *C. paspali*. *Neotyphodium coenophialum, Epichloë festucae* and *E. glycines* shared a different gene arrangement of 11 EAS genes. *Epichloë brachyelytris* isolate E4804 had putatively functional genes only for the initial four enzyme steps of the pathway.
Ergot alkaloid gene expression studies in a grass-endophyte association
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Phytopathology 101:S54
Many epiphitic endophytes (Epicluboe and Neoeytthodium spp.) are systemic symbiots of cool-season grasses, providing protection against verticrude and invasive herbivores by producing mycotoxins. Among these compounds are the ergot alkaloids (EA) responsible for livestock toxiosis. The outcome of the EA pathway is a complex profile of alkaloids consisting of the pathway end product and several intermediates that can accumulate at levels comparable to the end products. These endophytes grow vegetatively throughout aerial tissues of infected plants without morphological differentiation. However, there is a possibility that fungal hyphae in different tissues have different gene expression patterns. To test this hypothesis, we conducted a reverse-transcription-quantitative PCR study of the N. lolii x E. typhina hybrid isolate Lp1 in perennial ryegrass to measure the expression of six EA biosynthesis genes (dnuA, easC, easD, easA, clmA, and lpsA) in grass pseudostems, center leaves, outer leaves, leaf blades, and developing seeds. For each gene, tissue samples from at least three individual plants were analyzed. Possible relationships between gene expression levels and ergot alkaloid profiles were examined. Within the same plant tissue all examined genes revealed similar expression profiles, whereas expression levels differed widely among different tissues. There was no definite correlation between the differences in gene expression and the profile of alkaloids accumulating in different tissues.

High levels of natural resistance against selected DM1 fungicides in populations of Fusicladosporium carphophilum but not Alternaria spp. from Arizona
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Phytopathology 101:S54
Scab caused by Fusicladosporium carphophilum and Alternaria leaf spot caused by Alternaria spp. are common and economically important diseases of alfalfa in California. To replace the oversold Quilos where widespread resistance has developed in both pathogens, newly registered DM1 fungicides have proven very effective in managing these diseases. Baseline sensitivity studies were conducted to aid in monitoring of fungicide sensitivities of field populations. F. carphophilum exhibited a wide and continuous range of sensitivities for difenoconazole (EC50 0.002 to 5.455 µg/ml, mean 0.283 µg/ml), metconazole (EC50 0.013 to 3.85 µg/ml, mean 0.496 µg/ml), and propiconazole (EC50 0.045 to 6.701 µg/ml, mean 0.755 µg/ml), whereas isolates sensitive to one compound were also less sensitive to the other compounds. Thus, many of the isolates were naturally resistant to these fungicides. All isolates of Alternaria spp. were determined to be sensitive against the three fungicides. EC50 values ranged from 0.007 to 0.076 µg/ml (mean 0.017 µg/ml) for difenoconazole, 0.014 to 0.224 µg/ml (mean 0.045 µg/ml) for metconazole, and 0.028 to 0.172 µg/ml (mean 0.084 µg/ml) for propiconazole. To maintain a high level of disease control with the use of these fungicides, resistance management with strict rotations of fungicides with different modes of action will need to be done in integrated programs.

Fluopyram fungicides for the control of diseases of horticultural and row crops
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Phytopathology 101:S54
Fluopyram is a new fungicide active ingredient in development worldwide by Bayer CropScience. Application for registration is pending with the Environmental Protection Agency with an expected registration summer or fall of 2011. In addition to Luna Privilege (fluopyram solo), premixes were developed with four other fungidal active ingredients: Luna Sensation (+ trifloxystrobin), Luna Experience (+ tebuconazole), Luna Tranquility (+ propiconazole), and Propulse (+ prothioconazole). The mixtures have demonstrated excellent crop safety and outstanding control of a broad range of major foliar and fruit diseases such as powdery mildew, brown rot blossom blight, early- and late blight, gray mold, anthracnose, and pink bollworm. Results are providing improved crop quality at harvest and during storage/transportation. Biological profile, efficacy trial results, resistance management/mode of action, and pending use labeling will be presented.

Bacteria associated with creeping benggrass (Agrostis palustris L.) disease syndrome in southern & southeastern United States during the summer of 2010
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Phytopathology 101:S54
During the summer of 2010, the Clemson University Commercial Turfgrass Research Unit received samples of benggrass putting greens from 15 courses from southern & south eastern United States. The samples were submitted on suspicion of Pythium infection. Additional samples were received and described as “healthy” turf. On the samples suspected of having disease, symptoms were varied from yellowing of lower leaves to wilt and dessication of entire plants. Microscopic observations revealed streaming of bacteria from both infected yellow leaves and newly emerging leaves. There was no evidence of Pythium oospores or zoospores. Following surface disinfection, bacteria were isolated by maceration of infected leaves in nutrient broth and streaking the suspension on nutrient agar (NA). Pure cultures of predominant bacterial colonies growing on plates were established on NA. Based on color, and morphology of bacteria on several media we were able to distinguish 16 different bacterial morphologies. Pathogenic testing of each culture was conducted on creeping benggrass (Agrostis palustris L.) cv. Penn G-2. There were 11 pathogenic isolates belonging to 10 different bacterial morphologies. Sequence analysis of 16S rDNA of pathogenic bacteria revealed the highest similarity (>98%) to Xanthomonas translucens pv poae, Acidovorax avenue subsp avenue, and a similarity of (>93%) to X. campestris pv campestris and X. oryzae pv oryzae for all the 11 pathogenic isolates.

Modelling of Guignardia pseudotrichemum maturation and ascospore dispersal in citrus orchards
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Phytopathology 101:S54
Ascospores are considered the most important inoculum source citrus black spot (CBS), caused by Guignardia citricarpa, but pseudotrichemum maturation and ascospore dispersal are inadequately studied. Guignardia ascospore trapping and concomitant weather data were obtained for three localities for three seasons (July through March from 2006 to 2009) in the Limpopo province of South Africa. Degre-days accumulated until first seasonal ascospore discharge (>10°C with 1 July as biofix; DDtemp), and DDtemp accumulated on rainy (rainfall >0.1 mm; DDrain) and moist days (vapour pressure deficit <5 hPa; DDvpd) were used in two Gompertz models to predict onset of ascospore dispersal: a temperature model [Event = exp(-exp(-(-2.725 + 0.004 × DDtemp)))] and a temperature/moisture model [Event = exp(-exp(-exp(-3.238 + 0.008 × DDvpd + 0.004 × DDtemp -0.009 × DDrain)))] (R² = 0.608 and 0.658, respectively). Both models predicted a delay in pseudotrichemum maturation in climates with colder winters and springs, while the temperature/moisture model predicted a further delay in drier seasons or climates. A Gompertz equation was also used to predict the proportion of Guignardia ascospores trapped (PAT) per season from DDtemp data accumulated on wet or moist days from the first seasonal ascospore discharge [PAT = exp(-4.096 × exp(-0.005 × DDwat2)]; R² = 0.908). These models can be used to predict the onset and dynamics of ascospore dispersal in climatically diverse regions.

Geminiviral (PHYVV and PepGMV) and cucumoviral (CMV) co-infection in chili pepper fields: The AC1 gene in PepGMV with a mutation with aminoacid change
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Phytopathology 101:S54
Emerging diseases in chili pepper (Capsicum annuum L.) are causing important agricultural crop losses in chili producing provinces of central part America. Symptoms exhibited by the affected plants consist of yellowing, dwarfing, or sprout death, scarce and small size fruits without commercial value. The object of this study is to identify the possible viral species as causal agents of these emerging diseases in chili pepper in producing areas in Zacatecas state, México, Lookalikes of nuclear acids hybridization (colony hybridization, Southern blot, tissue printing), rolling-circle amplification of nucleotide sequences, sequencing and sequence alignment inGenBankand immunology assays (ELISA) where used. Results show the presence of the PHYVV and PepGMV (Begomovirus) with high homologies to isolates.
previously reported in other latitudes in this country. The immunology assays with monoclonal antibodies indicate the presence also of the CMV (Cucumovirus). In the AC1 gene of the PepGMV a nucleotide change of C instead of A was found. This mutation impact the central domain of the respective protein, where the aminocacid N (Asn) is changed by H (His). In conclusion, in chilli pepper crop with yellowing and dwarfing symptoms in this latitude, symptoms of virus are present, the PHYVV, the PepGMV and the CMV; in the PepGMV a single nucleotide mutation was found with impact in the central domain of the Rep protein.

**Citrus cybrid response to biotic stress caused by Xanthomonas citri subsp. citri**

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Phytopathology 101:S55

A cybrid is an asymmetric hybrid that contains the nucleus of one parent in combination with the mitochondrion and/or chloroplast of the cytoplasm donor parent. Twenty cybrids of highly susceptible Red grapefruit (RG, Citrus paradisi) and the more tolerant Valencia orange (VO, Citrus sinensis) as the cytoplasm donor, were screened for their susceptibility to Xanthomonas citri subsp. citri (Xcc). Tolerance inherited from VO appeared to be quantitative based on an intermediate lesion phenotype in selected cybrids. In contrast to the callus-like lesions typical for susceptible RG, lesions were more necrotic for VO and the cybrids. This lesion phenotype indicated cell death arrested the proliferation of Xcc. Populations of Xcc at 14 days post inoculation in cybrids (7.2 Log cfu), were similar to VO (7.6 Log cfu) and one log unit lower than RG (8.4 Log cfu). Expression of genes related to host pathogen interaction in VO and cybrids from VO and from RG. The contrasting pattern suggested a differential interaction of genes from the nucleus with the mitochondria and chloroplast genes from the cytoplasm donor. Mitochondria and chloroplasts have a central role in stress and programmed cell death signaling. The response of cybrids to Xcc may be expressed at different levels depending on whether mitochondrial and/or chloroplast genomes are transferred in the cybridization process.

**Temperature and fungal isolate influence canker development in black walnut caused by Geosmithia morbida**

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Phytopathology 101:S55

Thousand cankers disease of black walnut ( Juglans nigra) is the result of aggressive feeding by the walnut twig beetle (Pityophthorus juglandis) and subsequent canker formation around galleries caused by Geosmithia morbida. We studied temperature effects and G. morbida isolate aggressiveness on canker development in black walnut. One-year-old trees were placed in growth chambers maintained at 20°C at night and either 25 or 30°C during the day. Each tree was inoculated with four different haplotype isolates (based on rDNA ITS sequence data) positioned on stems in a Latin square design. There was no effect (P > 0.10) of inoculation position on canker development but cankers were larger (P < 0.05) at 25°C compared to 30°C six weeks after inoculation. All isolates tested caused cankers, although in two experiments an isolate collected from Arizona walnut (J. major) in Arizona resulted in slightly smaller (P > 0.05) cankers than the other isolates collected from black walnut. Furthermore, all canker areas were smaller in experiments in which trees were entering dormancy or were fully dormant (i.e. they had moliliated by late fall in the greenhouse) prior to inoculation in growth chambers. These data suggest that black walnuts are more susceptible to canker development when they are actively growing.

**Genetic based population analysis of the nucleocapsid protein of Tomato spotted wilt virus isolates in New Mexico**

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Phytopathology 101:S55

Tomato Spotted Wilt Virus (genus Tospovirus; family Bunyaviridae) is an important pathogen of many ornamental, greenhouse and agronomic crops worldwide. Over one thousand different plant viruses have been characterized and Tomato Spotted Wilt Virus (TSWV) ranks in the top ten most economically damaging viruses in the U.S. For this project, we developed a gene for green fluorescent ORSV (GFP) and is stably maintained in R. solanacearum cells without selection pressure. Bacteria harboring the plasmid can be tracked in plants by visualizing GFP fluorescence. For real-time monitoring of bacteria in plants, tomato seedlings were grown on agar medium and bacterial suspension was applied to the root apex. Aseptic inoculation of plants grown on solid agar medium eliminates the effects of other bacteria in the soil. In susceptible tomato cultivars, strong GFP fluorescence was observed in hypocotyls and lateral roots as well as the taproot. In resistant cultivars, however, GFP acid difference and the predicted steric implications to the secondary protein structure were examined. The viral population among the major host groups was relatively homogeneous and the population of all TSWV isolates from New Mexico was 98.8% similar. When the viral population in New Mexico was compared to other TSWV isolates from around the world, including known resistance breaking strains, a similar homogeneity resulted. Most of the amino acid substitutions observed were conserved changes that had little or no effect on the final protein product.

**Diapause in northern corn rootworm (Coleoptera: Chrysomelidae)**

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Phytopathology 101:S55

Diabiotic corn rootworms are prominent pests of maize and have adapted to both cultural and chemical management methods. In response to a widely used corn-soybean crop rotation in the U.S. Corn Belt over several years, northern corn rootworm (NCR) populations adapted by increasing the proportion of eggs that diapause over two winters instead of one. The frequency of eggs diapausing for two years increased with time and prominence of the maize-soybean rotation in the landscape. We investigated the pattern of inheritance of egg diapause duration in relation to male and female parent phenotypes for diapause duration. We collected NCR as pupae from a maize field that had been in a maize-soybean crop rotation for several years. We sexed the pupae and maintained them individually. We also collected pupae from a NCR lab colony that had been selected for one year diapause for several generations. We established reciprocal F1 families from the extended diapause (ED) and one-year diapause (D) lines. Eggs obtained from the females were provided two overwintering periods, one each for five months at 8°C. Eggs were allowed to hatch at 25°C for 45 days after each overwintering period. Eggs obtained from ED females had a significantly higher proportion of eggs with the ED trait compared to eggs obtained from D females (P {eq}\leq {eq} 0.015).

With a strong genetic influence on diapause duration in NCR, we can begin selecting for a non-diapausing line to facilitate research on this important pest.

**Generation and affinities with antigen of single chain variable fragment antibody against Odontoglossum ringspot virus from phage display library**

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(1) NCYU, Chia-yi, TAIWAN; (2) TNDAS, Chia-yi, TAIWAN

Phytopathology 101:S55

Odontoglossum ringspot virus (ORSV) is one of the commonest viral pathogens of the cultivated orchids. In this experiment a set of ORSV-specific oligonucleotide primers were designed from the region of the coat protein (CP) gene of ORSV. The ORSV CP gene was cloned into the protein expression bacterial plasmid vector of the gutathione S-transferase (GST) fusion protein expression system. The recombinant ORSV CP was injected into mouse to induce immune response of the animal. The cDNAs of VH and VL of ORSV antibody genes were obtained by using reverse transcription polymerase chain reaction from the total RNAs that were extracted from the spleen cells of immunized mouse. ScFv (single-chain variable fragment) library of ORSV were constructed with gene splicing by overlap extension. Thirty seven scFvs were selected from ORSV-scFv library following three rounds of affinity selection with ORSV CP as an antigen that was expressed in bacteria. Four scFv antibody have specific binding reaction against ORSV CP and were expressed in bacteria. Four scFvs were selected to obtain scFv antibody against ORSV CP. Comparing the sensitivity between scFv antibodies and ORSV polyclonal antibody titested in enzyme linked immunosorbent assay (ELISA) to detect ORSV in leaf extracts of diseased Phalaenopsis plants. Unfortunately, the affinity between scFv antibody and ORSV was weaker than that between polyclonal antibody to the same antigen. The results highlight the potential of applying the scFv antibodies in the diagnosis of virus diseases.

**Monitoring behaviors of Ralstonia solanacearum cells by GFP labeling during infection process to plant cells**

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Phytopathology 101:S55

Growth and movement of Ralstonia solanacearum harboring the phage-modified plasmid were monitored using tomato seedlings and tobacco coleoptile by real-time fluorescence. The plasmid contained a gene for green fluorescent ORSV (GFP) and is stably maintained in R. solanacearum cells without selection pressure. Bacteria harboring the plasmid can be tracked in plants by visualizing GFP fluorescence. For real-time monitoring of bacteria in plants, tomato seedlings were grown on agar medium and bacterial suspension was applied to the root apex. Aseptic inoculation of plants grown on solid agar medium eliminates the effects of other bacteria in the soil. In susceptible tomato cultivars, strong GFP fluorescence was observed in hypocotyls and lateral roots as well as the taproot. In resistant cultivars, however, GFP...
fluorescence was rarely observed on lateral roots. The difference may be due to gaps between the taproot xylem and the lateral root xylem. It appears that *R. solanacearum* cells require a long period of time to move between xylem gaps in resistant cultivars. We also observed that bacterial growth was suppressed in the hypocotyl and stem of the seedlings of resistant cultivar. Our results show that this monitoring system can be used to assess bacterial pathogenicity. For further study, we made strains of *R. solanacearum* lacking cell wall degrading enzymes (CWDE) and investigated the effects using BY-2 cells.

Phylogenetic background of Japanese *B. cinerea* isolates resistant to benzimidazoles, dicarboximides, and other fungicides

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Phytopathology 101:S56

*B. cinerea*, a causal agent of gray mold, is one of pathogens showing a high risk of development of fungicide resistance. We previously identified four types of benzimidazole-resistant mutations in beta-tubulin *BenA* gene, three types of dicarboximide-resistant mutations in histidine kinase *BcOS1* gene, and a QoI-resistant mutation in cytochrome b (*cyt b*). We analyzed genetic background to investigate the origins of fungicide-resistant strains in Japan. Eighty three isolates were divided into three groups by microsatellite primed-polymerase chain reaction (MP-PCR) and further divided into 21 groups by the PCR-restriction fragment length polymorphism (RFLP) at nitrate reductase, *ADP* ATP translocase, and ATP synthase genes. Major dicarboximide-resistant *BcOS1* and benzimidazole-resistant *BenA* mutations were found in various strains suggesting that these mutations occurred in various strains independently during long term use of these fungicides. The dicarboximide-resistant isolates with *BcOS1* mutation had low genetic diversity. Interestingly most of these isolates showed fenhexamil resistance. Although QoI-resistant isolates were present at low rate in fields, six QoI-resistant isolates were divided into three different genotypes. We will discuss why *B. cinerea* has high genotypic diversity and also how fungicide resistant strains raised and spread in population.

New Phomopsis species identified from wood cankers in eastern North American vineyards

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Phytopathology 101:S56

Phomopsis cane and leaf spot, caused by the Ascomycete fungus *Phomopsis viticola*, is a destructive fruit and foliar disease in eastern North American vineyards. The pathogen typically attacks green tissues, but can also cause wood cankers, presumably due to infection of pruning wounds, as is the case for most canker pathogens of grape (e.g., *Eutypa lata*). If pruning wounds are an infection court for *P. viticola*, then controls for preventing infection of green tissues may not prevent pruning-wound infection. Accordingly, we surveyed the Phomopsis community (teleomorph *Diaportha*) from wood cankers in vineyards of the northeastern U.S. (CT, MA, MD, MI, NH, NJ, NY, OH, RI, VA, VT) and southeastern Canada (Ontario, Quebec), and evaluated the susceptibility of pruning wounds to infection by the Phomopsis species present. Numerous wood cankers, containing growth on potato dextrose agar, and phylogenetic analyses of nuclear loci (*rDNA* internal transcribed spacer region, elongation factor subunit 1-alpha, actin), to identify *P. viticola* from wood cankers and two new species not previously reported from grape: *Diaportha eres* and a species with DNA sequences identical to isolates identified as *P. fukashii* in Japan. Pathogenicity tests on *Vitis labruscana* 'Concord' and *V. vinifera* 'Chardonnay' in Geneva, NY demonstrated that pruning wounds of both are susceptible to infection by strains of all three *Diaportha/Phomopsis* species.

Assessment of prescription programs using Peanut Rx for management of peanut diseases

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Phytopathology 101:S56

Peanuts in the southeastern U.S. are affected by numerous fungal diseases and management programs often include 7 fungicide applications per season. Since 2007, a risk index, Peanut Rx, has been updated by researchers from the University of Georgia, the University of Florida, and Auburn University. From points assigned to production variables, disease risk is described as low, moderate or high. Prescription fungicide programs have been developed appropriate for risk (4, 5, or 7 applications/season). Studies were conducted at 3 sites in Georgia in 2010 to assess prescription programs that included flutolanil, propiconazole, tebuconazole + prothioconazole, tetraconazole, tebuconazole, azoxystrobin, thiophanate methyl, and chlorothalonil. Plots were planted to 'Georgia-06G' and maintained according to recommendations from Cooperative Extension. Fungicides were applied at timings appropriate for prescription programs. Severity of leaf spot diseases was reduced in all fungicide programs as compared to the untreated control; incidence of southern stem rot tended to be significantly lower in fungicide programs than in the untreated control. Yields in treated plots were numerically, often significantly, greater in treated versus untreated plots. Differences in control of stem rot and yields were not different within related prescription programs, i.e. azoxystrobin programs; however leaf spot severity was frequently greater in plots sprayed 4 times versus 7 times.

Detection of Phomopsis sclerotioides in commercial cucurbit field soil by a nested time-release PCR-based technique

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Phytopathology 101:S56

A PCR-based molecular technique to detect *Phomopsis sclerotioides* in soil was developed using a species-specific primer pair. Three PCR techniques were combined to improve detection sensitivity: nested PCR using the primer pair ITS1 and ITS4, time-release PCR with two different polymerases (Taq and AmpliTaq Gold polymerases), and fluorescent PCR to obtain fluorescent-labeled PCR products that can be analyzed by capillary electrophoresis. Using these techniques, soil samples collected from 241 commercial cucumber or melon fields in Akita Prefecture in Honshu, Japan, were diagnosed to detect the pathogen. Disease incidence had not been observed in any of the fields. The pathogenic fungus was detected in soil samples collected from 30 fields but was not detected in samples from 207 fields. Samples from four fields remained inconclusive. In nine of the 30 fields showing a positive diagnosis, disease incidence had been confirmed or the pathogen had been isolated. The results demonstrate that the pathogen can be detected in cucurbit fields in which visible disease symptoms have not appeared. In order to prevent the invasion of the pathogen or delay its spread among fields in cucurbit-growing regions by periodic monitoring of the field soil, a highly sensitive detection technique is required. The technique developed here is practicable for this purpose.

Species-specific detection of *Mycosphaerella* spp. as classical biological control agents for *Fallopia japonica* (Japanese knotweed) by PCR assay

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Phytopathology 101:S56

*Mycosphaerella polygoni-cuspidati* has potential as a classical biological control agent for the invasive weed, *Fallopia japonica*. It also suggested that a novel species, *M. shimabarensis*, which has been isolated only from Shimabara, Nagasaki Pref., Japan, can be synergists with *M. polygoni-cuspidati* based on the results of promoting the disease severity. For direct, rapid and specific detection of *M. polygoni-cuspidati* and *M. shimabarensis* after introduction to the fields in the UK, specific primer sets were designed based on the sequences of *rDNA*-ITS region. PCR products of approximately 300 and 450 bp were obtained only when DNA extracted from mycelial fragments of *M. polygoni-cuspidati* and *M. shimabarensis* were used. No amplification was observed from other *Mycosphaerella* spp. and fungal endophytes isolated from *F. japonica*. Using the primer pairs, both isolates were specifically detected from naturally infected plants by *M. polygoni-cuspidati*. Therefore, the primer pairs can be useful for specific detections of both *M. polygoni-cuspidati* and *M. shimabarensis* distributed as mycelial fragments of *M. shimabarensis* detected by specific primers designed in this study was also investigated. A PCR product specific to *M. shimabarensis* was amplified from the genomic DNA extracted from the lesions but not from healthy area of diseased leaves. These results indicated that *M. shimabarensis* strongly associated with *M. polygoni-cuspidati*.

Evaluation and popularization of integrated pest management module in onion

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Phytopathology 101:S56

The major cause for low productivity of onion is the severe incidence of pests and diseases leading to yield losses up to 30 per cent and in severe outbreak situations the total crop loss. Five different onion IPM modules were evalu-
During characterization of a panel of Phytopathology 101:S57
Moscow, ID, U.S.A.
from Brazil unusual isolates were typed as PVY O-negative with monoclonal antibodies MAb2 (Agdia) and SASA-O (SASA). Some of these isolates were identified reacting normally to another PVY N-specific antibody 1F5 (Agdia). All these PVYNTN by RT-PCR assay were found non-reactive to a PVY N-specific
viride
tated in two seasons during 2008–09 to select the best module for popu-
targets were introduced two newly detected populations of Fusarium graminearum collections from
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Phytopathology 101:S57
Detailed analysis of extensive U.S. Fusarium graminearum collections from the past ten years has revealed surprising substructuring of the species into several populations that are characterized by their genetic coherence, their geographic distributions and by distinct phenotypic characteristics. Here, we introduce two newly detected populations of F. graminearum, the Northland and the Arkansas population. The Northland population mainly has been detected in Minnesota, and to a lesser extent in North Dakota, Wisconsin and South Dakota. This population appears to be endemic to native grass populations in Minnesota and its current distribution indicates outward radiation into agricultural areas. A subset (30%) of this population does not produce the common trichothecene mycotoxins, but still maintains aggressiveness on wheat. Arkansas appears to be a melting pot. Four populations are present in the state, with the widespread MW15ADON population and the newly detected Arkansas population each constituting about 30% of the total population. While the Southern Louisiana and the Gulf Coast populations also were encountered, a large percentage (ca. 30%) could not be assigned with a high degree of certainty. Unaligned isolates may be the result of interbreeding among populations in this region. All three trichothecene types were identified in the MW15ADON and Arkansas populations, but the proportions were different with 15ADON at 95% and 50%, 3ADON at 4% and 50%, and NIV at 1% and 4%, respectively.
A novel type of Potato virus Y recombinant genome
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Phytopathology 101:S57
During characterization of recombinant Potato virus Y (PVY) isolates collected in Brazil, two isolates, PVY-AGA and PVY-MON, were identified as a new type of PVYNTN recombination pattern. Whole genome sequencing analysis revealed that PVY-AGA and PVY-MON represented recombinants between two novel parent genomes, PVYNTN and PVY-NE11. Specifically, new recombinants had an ordinary PVYNTN genome structure for approximately 6.7-kb from the 5'-end of the genome, while the 3'-terminal 3.0-kb segment had two fragments of NE11 sequence separated by another small NTN fragment. Only PVY-AGA induced vein necrotic reaction in tobacco, both PVY-AGA and PVY-MON isolates did not induce hypersensitive resistance (HR) in potato cultivars carrying Ns, Nc, or (putative) Nc genes. An ordinary PVYNTN isolate PVY-AST induced systemic HR in cultivar Maris Bard carrying a putative Nc gene. All three isolates, PVY-AGA, PVY-MON, and PVY-AST, induced typical potato tuber necrotic ringspot disease in a susceptible potato cv Yukon Gold under greenhouse conditions. In a standard multiplex RT-PCR assay, PVY-AST, PVY-AGA, and PVY-MON were all typed as ordinary PVYNTN isolates, consistent with the presence of two prominent recombinant junctions in their genome, characteristic of an ordinary PVYNTN strain. Ability of these new PVY recombinants to overcome resistance genes in potato producing mild or no foliar symptoms presents a significant threat posed by these isolates to seed potato production areas.
Temperature-dependent development and reproduction of the whitefly Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae)
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Phytopathology 101:S57
Trialeurodes vaporariorum is a serious pest in sub-tropical regions and vector of the potato yellow vein virus. A profound understanding of the temperature-dependent population growth potential is important for understanding pest population dynamics and to design effective pest management strategies. The development and mortality of immature life stages, and reproduction and longevity of T. vaporariorum was studied at constant temperatures of 10, 15, 18, 20, 25, 28 and 32°C. Optimum temperature for development was between 27–28°C for the immature developmental stages. No complete development was observed at 10°C and 32°C. Survival time of adults was shortest at 15°C and 28°C with highest fecundity at 20°C. The data was used to establish functions for temperature-dependent development, mortality and reproduction. The established functions were used to compile a temperature-driven phenology model for T. vaporariorum, and life table parameters were simulated over a range of temperatures. The model was validated by comparing simulated life table results with life tables constructed under controlled daily fluctuating temperature (between 5.81–35.27°C). The model will be used in pest risk assessments studies and for predicting within year population growth potentials. Moreover, the information on the pest age-stage structure and distribution under specific field conditions will be useful for adapting IPM strategies.
Studies on *Peaнтus bud necrosis virus* affecting tomato in India
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Phytopathology 101:S58

*Peaнтus bud necrosis virus* (PBNV; genus *Tospovirus*, family Bunyaviridae) is a significant constraint to production of tomato by subsistence farmers in India. The IPM-CRSP of the USAID (2001–2005) has funded a project to implement ecologically-based IPM strategies for sustainable management of the virus. For this purpose, we have conducted farmer-participatory field trials in select locations of Tamil Nadu State in India and evaluated the performance of selected tomato cultivars and hybrids against natural infection of PBNV. Although none of the entries showed resistance, the data obtained from these trials identified cultivars and hybrids exhibited field tolerance with higher fruit yield compared to susceptible materials. The chemical composition of tomatoes harvested from PBNV-infected plants indicated significantly less amounts of lycopene, β-carotene, vitamin A, zinc, total sugars and carbohydrates suggesting that virus infection affected nutritive quality of the fruit. Studies on PBNV spread in new plantings indicated that virus-infected seedlings from commercial nurseries serve as a source of inoculum for secondary spread of the virus in the field. Roguing of virus-infected tomato seedlings during and soon after transplanting significantly reduced disease incidence leading to higher income for farmers. A combination of growing tolerant cultivars and roguing of virus-infected seedlings are being validated in IPM packages for mitigating impacts of PBNV for the benefit of farmers.

Differences in responses and protein profiles of soybean near isogenic lines (NILs) to *Phakopsora pachyrhizi* inoculation
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Phytopathology 101:S58

Asian soybean rust, caused by *Phakopsora pachyrhizi*, was first discovered in continental U.S. in late 2004. This pathogen has the potential to cause severe yield losses as all U.S. commercial soybean varieties are susceptible. In this study, ten Near Isogenic Lines (NILs) of three different populations were evaluated for differences in resistance to infection by *P. pachyrhizi* (Louisiana isolates). These lines, which had previously been evaluated against Florida soybean rust isolates, were evaluated in both growth chambers using detached leaves and under greenhouse conditions. For each line, sixteen plants were evaluated at R1 stage through inoculation with 200 µl of spore suspension (3 × 10^6 spores/mL) per leaf on the upper surface. For detached leaf assay, soybean leaves at R1 stage were inoculated in the same manner. Fifteen days after inoculation, plants in greenhouse and detached leaves in growth chamber were evaluated for lesion appearance, pustule formation, and pustule eruption density. There was a significant difference among NILs in response to *P. pachyrhizi* infection in growth chamber and greenhouse conditions. Some of these lines are currently being compared for protein profile differences with and without soybean rust inoculation to identify potential proteins involved in soybean resistance to rust infection.

Development of loop-mediated isothermal amplification (LAMP) assays for the detection of Plum pox virus
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Phytopathology 101:S58

Plum pox virus (PPV) is a devastating viral disease of stone-fruitin worldwide, and caused severe economic and production losses in many European countries. Molecular-based detection methods for PPV using conventional PCR, multiplex PCR and real-time PCR had been developed and applied. However, such methods require expensive instruments and a long time for detection. A more rapid, efficient and practical method, the reverse transcription-loop-mediated isothermal amplification (RT-LAMP) method was expected for the on-site detection of Plum pox virus. The PPV-LAMP specific primers were designed according to the sequences for coat protein gene of PPV-D and PPV-M. Amplification products were detected by agarose gel electrophoresis, checked up with the naked eye and by UV irradiation using SYBR Green I, lateral flow devices (LFD) and a real-time turbidimeter. Accordingly, a typical ladder-like pattern on the gel electrophoresis, a visible green after adding SYBR green I and two clear lines on LFD were observed in all positive samples. The results of real-time monitoring showed that the detection limit of the PPV-LAMP assay was 1.6 × 10^2 copies/µl in less than 30 min, and was approximately 100 times higher sensitive than that of the conventional PCR. The results above suggested that the LAMP technique was fit for the on-site detection of plum pox virus. With the improvement of the method, it might be extended to the survey and inspection for the purpose of quarantine.

Identification of biochemical function of *Agrobacterium T*-complex recruiting protein VBp
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Phytopathology 101:S58

A. tuneciacens can transfer a section of DNA from Ti plasmid to the host plants, resulting in crown gall tumor disease. The DNA was transferred in the form of a single-stranded DNA-protein complex (referred to as T-complex) and through the type IV secretion system (T4SS) located at both ends of the *Agrobacterium* cell. A recently identified protein that can bind to VirD2 (VirD2-Binding Protein, VBp) is proved to play a role of recruiting T-complex to the T4SS, and defined as recruiting protein of *Agrobacterium* T-complex. *Agrobacterium* contains three homologous genes that can encode three varieties of VBp proteins, named VBp1, VBp2, and VBp3. Motif search showed that VBps contain a HEPT domain and a nucleotide/transferase/transferase domain. To identify the biochemical function of VBps, VBp1 was expressed as His-tagged-VBp1 fusion protein and purified by affinity chromatography. The purified fusion protein was partially renatured at low temperature, and then was added to a solution that contains eight nucleotide triphosphates. After incubating for a certain period of time, the composition of nucleotides in the solution was analyzed by using HPLC to check whether any of these eight nucleotides was bound to VBp1. The results showed that His-tagged-VBp1 could hydrolyze all the eight nucleotides in different rate. Further quantitative calculations showed that the hydrolysis ability of VBp1 fusion protein to nTPs is significantly higher than that to the dNTPs.

RNA-seq analysis of potato tuber transcriptome dynamics in response to the late blight pathogen *Phytophthora infestans*
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Phytopathology 101:S58

Cultivated potato is the world’s number one non-grain food commodity. The late blight pathogen *Phytophthora infestans* is a notorious plant destroyer with the capacity to attack both potato foliage and tubers. Importantly, potato resistance against late blight does not guarantee tuber resistance. Our research documents that *RB*, a broad spectrum late blight resistance gene, has the potential to mediate both foliar and tuber resistance against late blight. To better understand *RB*-mediated tuber blight resistance, we conducted a high-throughput RNA-seq study to examine potato defense mechanisms. Eight potato RNA samples from *+RB* and *−RB* lines at 0, 24, 48 hours post pathogen inoculation were sequenced using Illumina GAIIx technology. Over 215 million cDNA sequence reads were generated. These represented 99% of known potato unigenes, and enabled a detailed analysis of tuber blight transcriptome dynamics. Groups of genes were identified that are candidate components of tuber blight resistance. Importantly, this preliminary study supports that the tuber and the foliage blight defense mechanisms are non-overlapping. An additional 42 potato RNA samples including *P. infestans*-challenged foliage and tuber samples, were subsequently sequenced using Illumina High-seq 2000 technology. The resulting ~300-600 million sequence reads will enable a more robust and fine-scale analysis of tuber blight transcriptome dynamics and a more detailed comparison between potato tuber and foliage blight defense mechanisms.

Prophages of “*Candidatus Liberibacter asiaticus*” and their distribution in southern China
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Phytopathology 101:S58

“*Candidatus Liberibacter asiaticus*” is a putative pathogen of citrus hlorosis disease (HLB) that is the causative agent of devastating citrus disease worldwide. Prophage is an important biological trait of bacteria including “*Ca. L. asiaticus*”. The role of prophages in bacterial virulence, environmental adaptation, strain specification and genome evolution has recently drawn strong interests in the HLB research community. In this study, 12 consecutive open reading frames in a “*Ca. L. asiaticus*” prophage/phae reported in Florida were used as a reference to examine for the presence of homologs in three different “*Ca. L. asiaticus*” strains in China. PCR analyses showed the amplification rates of 83.3% (10/12) for strain YN835, 33.3%(4/12) for strain GDws231, and 83.3%
T1 and T2 recorded no mortality, but there was a reduction in plant height and F. oxysporum there was mortality of plants: in T3, 87.5%, 59 days after where R. necatrix was inoculated and there was interaction with O. stenoceras oxysporum + R. necatrix; T8 = Control. Inoculation was done by sp. vacara variety on Manetti pattern were sown; eight treatments were pathogenicity tests. In 12-inch pots with disinfected substrate plants of Rosa & Nannf. were found. Thus the objective of the study was to conduct Fusarium oxysporum Schlechtend and O phyostoma stenoceras (Robak) Melin recent times a root rot and subsequent death problem of this plant has Rosa cultivation in greenhouse is important in Mexico (south of the State of Colegio de Postgraduados, Fitopatología, Montecillo, MEXICO (1), D. Nieto-Angel (2) R. GARCÍA-VELASCO (1), J. G. González-Díaz (1), T. Castañeda-Martínez stenoceras in white rot of Rosa sp. population the UI-PCN Laboratory is now producing an adequate number of cysts from the Idaho population for research on control and eradication of this pest in Idaho.

Interaction of Rosellinia necatrix, Fusarium oxysporum and Ophyostoma stenoceras in white rot of Rosa sp.

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Rosa cultivation in greenhouse is important in Mexico (south of the State of Mexico) mainly for ornamental purposes; 663 ha are currently cultivated. In recent times a root rot and subsequent death disease of this plant has accentuated. By conducting causal agent isolates, Rosellinia necatrix Prill, Fusarium oxysporum Schlechtend and Ophystoma stenoceras (Robak) Melin & Nannf. were found. Thus the objective of the study was to conduct pathogenicity tests. In 12-inch pots with disinfected substrate plants of Rosa sp. vacara variety on Manetti pattern were sown; eight treatments were established: T1 = Ophystoma stenoceras; T2 = Fusarium oxysporum; T3 = Rosellinia necatrix; T4 = O. stenoceras + F. oxysporum; T5 = F. oxysporum + R. necatrix; T6 = O. stenoceras + R. necatrix; T7 = O. stenoceras + F. oxysporum + R. necatrix; T8 = Control. Inoculation was done by incorporation of 20 g of sp. vacara variety on Manetti pattern into 125 mL of a suspension of 10X6 conidia/mL of Ophystoma stenoceras and 125 mL of 10X6 conidia/mL of Fusarium oxysporum. The results showed that in treatments where R. necatrix was inoculated and there was interaction with O. stenoceras and F. oxysporum there was mortality of plants: in T3, 87.5%, 59 days after inoculation (dai); T6, T5 and T7 recorded 62.5, 56.3 and 66.7% mortality at 63.2, 59.4 and 60 (dai), respectively; T4 suggests antagonism between them; T1 and T2 recorded no mortality, but there was a reduction in plant height compared to the control.

Population structure and genetic diversity of Sclerotinia minor from peanut research plots in Oklahoma

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Sclerotinia minor is the causal agent of Sclerotinia blight, a disease that significantly reduces peanut (Arachis hypogaea) productivity. This study analyzed the diversity and population structure of Sclerotinia minor from Oklahoma. Isolates were obtained from infected stems of peanut plants from four lots at the Oklahoma Agricultural Experiment Station. Isolates used for Sclerotinia blight resistance screening in peanuts, collected between 1981 and 1993, were also included in the analysis. Inter Simple Sequence Repeat (ISSR) fingerprinting was used for evaluation of the population structure and genetic diversity of the S. minor sample. Of a total of 50 fragments amplified, 38 were polymorphic (76%). AMOVA identified significant genetic differences within the sample (ΦPT = 0.091; p = 0.001), of which 9.1% of the genetic variation exist between lots and the other 90.9% is distributed within lots. Nei’s genetic distance, GST, and PCO revealed close relationships between the populations from lots used for peanut breeding and isolates used for disease resistance screenings, with little although significant differentiation between plots. However, isolates collected from chemical testing plots were significantly different from all the others. Our results confirmed the validity of the current isolate panel for peanut germplasm Sclerotinia blight resistance screening. New S. minor genotypes were identified that could be of value for peanut breeding programs in the future.
thrips and their parasitoids from Africa is difficult. Absence of identification tools has contributed negatively to the invasion and widespread distribution of thrips like Western Flower Thrips, chilli thrips, corn thrips etc. To facilitate early and effective diagnosis of the native and invasive thrips and their parasitoids a user-friendly LucID 3.5 thrips identification system is under development. Detailed surveys on the thrips and their parasitoids in the region were undertaken and a geographic database was developed at ipicie. Based on the above surveys and the published literature, a list of over 90 thrips species including the predatory thrips as well as terebrantian and tubelliferan pest thrips are included in the key under development. A LucID sub-key to 8 eupholid thrips parasitoids belonging to the sub-family Entedoninae from Africa is under development. Biogeographic information, online occurrence maps and IPM options for the pest thrips are innovative features of the East African LucID key. Such a key could benefit quarantine authorities, extension functionaries and entomologists in the region for effective and timely identification of the thrips and their natural enemies. A snapshot view of the key and its features and future plans on the updates will be presented.

Development of a forecast model for the carpopgenic germination of Sclerotinia sclerotiorum Sclerotinia sclerotiorum causes annual white mold epidemics in several Brazilian regions. The aim of this study was to develop a forecasting model for the prediction of S. sclerotiorum sclerotia, in order to use it as part of a system for disease prediction and in the development of disease risk maps. For this purpose, an experiment was carried out to determine the effect of temperature and soil moisture on the carpopgenic germination of the pathogen’s sclerotia. Sclerotia were submitted in the laboratory at different temperatures (10, 13, 15, 18, 20, 23, 25 and 30 degrees C) and soil moisture (61, 65, 68, 75, 82, 89, 95, 100, 105, 110, 115 and 120% of field capacity, FC). The experimental units were composed of 20 sclerotia placed on 150 g of soil in 500 g plastic containers. The experiment was carried out in a randomized block design in a 8 × 11 factorial arrangement with four replications. Germination was initiated after 32 days of incubation at temperatures between 13° and 20°C, with humidity exceeding 68% of FC. The germination percentage was higher at 15°C and 40 days of incubation. A logistic regression model was used to estimate the probability of germination of sclerotia as a function of moisture and soil temperature. According to the model, temperatures between 15° and 17°C and humidity close to 100% of DC are more favorable for carpopgenic germination of S. sclerotiorum sclerotia.

Biological control of fire blight disease Erwinia amylovora under field condition of Karaj, Iran Fire blight (Erwinia amylovora) annually causes devastating damages on pear and quince crops in Iran and is in progress to the east. Due to efficiency of chemical controls and limitation of antibacterial usage, biological control could be an important method to reduce the disease damages. This research aimed at biological control of E. amylovora by using epiphytic bacteria, Pseudomonas fluorescens strain E10; Pantoea agglomerans strain Abp2; Pseudomonas putida strain E11 and Serratia marcescens strain Kgh1. The antagonists were isolated from pome tree rhizosphere and identified by morphological, biochemical, and physiological tests, as well as nucleotide sequence analysis of 16S-rRNA gene. The selected antagonist isolates were applied twice at 20% and 80% full bloom on a semi-susceptible pear cultivar ‘Shah-Mieveh’ in Karaj region in Iran. The results showed 46.87% disease severity in control plants, while the antagonistic bacteria reduced the disease symptoms between 23 to 50.2%. The most disease inhibition was belonging to P. agglomerans strain Abp2 and least disease inhibition was showed in S. marcescens strain Kgh1 in orchard trials.

Evaluating the spread of potato powdery scab in storage A. J. GEVENS (1), B. J. Webster (1), R. A. Clark (1) (1) University of Wisconsin, Madison, WI, U.S.A. Phytopathology 101:S60 Powdery scab, caused by the phialidicorin pathogen Spongospora subteranea, is a potato disease that has become a great concern in potato-growing regions of North America. Persistent cystosori can survive in soil for more than 6 years and their ability to cause disease is promoted by cool, moist weather and poorly drained soil conditions at tuberization. Cystosori are created in scab lesions that erupt through periderm and can cause infection at and post-harvest. In this 2-year study we investigated the spread of powdery scab from field-infected tubers to asymptomatic tubers in storage at the University of Wisconsin Hancock Storage Research Facility. In 2009, after 82 days in storage, 8 treatments composed of serial distributions of symptomatic and asymptomatic tubers resulted in 76–100% infected tubers. All treatments resulted in correlation between powdery scab infection and tuber desiccation. In 2010, the disease-spread experiment at 45 days resulted in low disease severity and slight desiccation. Additional treatments in 2010 included ambient ozone and phosphorous acid salts for limiting spread of infection in storage; no significant differences in control were observed at 45 days. Variable results between years may be resolved with extended ratings out to 90 days in storage. Due to the longevity of this pathogen in soil and limitations in management, it is critical that we better understand the role and risk of powdery scab in production and storage.

Evaluating the efficacy of fungicide programs for the control of potato early blight in the central sands of Wisconsin A. J. GEVENS (1), K. M. Cleveland (1), J. Dobbs (1), R. A. Clark (1) (1) University of Wisconsin, Madison, WI, U.S.A. Phytopathology 101:S60 Potato early blight is a perennial and potentially destructive disease caused by the fungus Alternaria solani. Appropriately-timed, effective fungicides are necessary to limit yield and quality loss. In 2010, we evaluated 38 fungicide programs for early blight control at the University of Wisconsin Hancock Agricultural Research Station on ‘Russet Burbank.’ Programs included an untreated control, conventional and organic grower standard programs, and newer chemistries, all replicated 4X and arranged in a randomized complete block design. Programs were initiated on 16 Jun and all other production inputs were commercial standard. Plots were treated every 7 days and evaluated for disease bi-weekly using a modified Horsfall-Barratt scale. Plots were machine-harvested on 22 Sep and tubers were graded for size and yield. No tuber early blight was observed and the specific gravities of tubers from top yielding programs were not significantly different. Programs that had the lowest Area Under the Disease Progress Curve values were the highest yielding. The highest yielding program was the Wisconsin conventional grower standard. Organic treatments were ineffective. Several newer chemistries and modified standard programs were effective. At this time, and due to the longevity of this pathogen in soil and in storage; no significant differences in control were observed at 45 days. Variable results between years may be resolved with extended ratings out to 90 days in storage. Due to the longevity of this pathogen in soil and limitations in management, it is critical that we better understand the role and risk of powdery scab in production and storage.

The ectomycorrhizal fungus, Sebacina vermifera, imparts drought tolerance to the bioenergy crop switchgrass (Panicum virgatum L.) S. R. GHIMIRE (1) (1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A. Phytopathology 101:S60 Drought is one of the most significant abiotic constraints limiting crop production worldwide. Mycorrhizal fungi have been shown to provide various fitness benefits to their host plants, yet their role in drought tolerance has been largely overlooked. This study investigates the drought tolerance imparted by an ectomycorrhizal fungus, Sebacina vermifera, to the important bioenergy crop switchgrass. An in vitro experiment revealed that switchgrass seedlings co-cultivated with S. vermifera performed well under mild drought stress, producing up to 63% higher biomass than mock-inoculated seedlings. Greenhouse experiment revealed that co-cultivated plants produced significantly taller plants (84%) with higher shoot (219%) and root biomass (162%) than mock-inoculated controls (P ≤ 0.01). Most significantly, co-cultivated plants under drought produced 120% and 156% higher shoot and root biomass, respectively, than mock-inoculated well-watered plants (P ≤ 0.01). The partial acquisition of primary and macrominerals (magnesium and sulfur) was significantly higher in co-cultivated plants compared to mock inoculated controls however, latter had equal or higher concentrations of these nutrients. The implications of these findings on the sustainable production of this important bioenergy crop switchgrass will be discussed.

Toward the development of integrated pest management (IPM) packages for tomatoes and other vegetable crops in West Africa R. GILBERTSON (1), M. Noussourou (2), K. Gamby (2), M. Osei (3), S. Miller (4), D. Pfiffer (5), C. Brewster (5), D. Mullins (5) (1) University of California-Davis, Davis, CA, U.S.A.; (2) Institut Economie Rural, Bamako, MALI; (3) Crops Research Institute, Kumasi, GHANA; (4) Ohio State University, Wooster, OH, U.S.A.; (5) Virginia Tech, Blacksburg, VA, U.S.A. Phytopathology 101:S60 Vegetable crop production is West Africa is typically done by smallholder farmers, many of which are women. Yield potential is limited by numerous
diseases, insect pests and weeds, as well as lack of access to improved varieties and technologies. The USAID-funded IPM-CRSP project in West Africa has made considerable progress in identifying disease and insect pest constraints on vegetable production. Through the plant virus global theme project, viruses causing diseases of a number of vegetable crops have been identified, including whitefly-transmitted viruses that limited tomato production in Mali and other West African countries. The international plant diagnostic network global theme has conducted a series of regional workshops on pest detection and diagnosis, and established an internet-based distance diagnostic network that allows for photographs of suspects pest problems to be sent to experts located throughout the world. This information together with results of surveys conducted about small farmers vegetable crop production practices are now being used to develop comprehensive IPM packages for key vegetable crops, including cabbage, potato and tomato. The package for tomato produced in the dry season that is presently being evaluated in Mali and Ghana will be presented. These IPM packages will hopefully be adopted by selected farmers and serve as models for other farmers and crops.

Identification of phytopathogenic fungi associated with giant miscanthus in Mississippi

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Phytopathology 101:S61

Giant miscanthus (Miscanthus x giganteus) is a tall, perennial C4 grass that is commercially produced as a bioenergy crop. In 2010, fungal diseases were observed in ten-year-old research plots in Starkville, Mississippi. A study was initiated to identify selected fungi associated with foliar symptoms of giant miscanthus clones ‘Freedom’ and ‘Illinois’ through classical techniques. Mature leaves showing symptoms of small, localized, elliptical lesions with straw-colored centers and reddish-brown margins were selected for fungal isolation. Lesions were excised from mature leaves and surface disinfested with NaOCl prior to being plated onto water agar. Fungal hyphal tips were transferred and incubated on water agar to facilitate identification based on reproductive structures. The predominant fungi isolated from foliar lesions of giant miscanthus were Alternaria, Bipolaris, Curvularia, Nigrospora and Phoma-like species. The presence of these fungi indicates possible pathogenic interactions capable of inciting disease on giant miscanthus. Confirmation of identity through DNA sequencing and pathogenicity of the fungi are being conducted. Further investigation is warranted to better understand the relationship between these fungi and giant miscanthus.

Foliar diseases identified on switchgrass in Mississippi

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Phytopathology 101:S61

Switchgrass (Panicum virgatum) is a warm season perennial grass native to areas in North America including the Black Belt of Alabama and Mississippi. Switchgrass is beneficial for wildlife habitat and is considered a primary renewable bioenergy source. Collections of foliar diseases that included leaf rust, anthracnose, and leaf spot from ‘Alamo’ switchgrass research plots at Mississippi State University were made in late summer 2009 and 2010. Symptomatic tissues were surface disinfested, plated onto water agar and incubated five days. Hyphal tips of fungi colonizing infected tissues were transferred to water agar. Light microscopy was used to identify fungi based on reproductive structures. Colletotrichum sp. was isolated from broad, elliptical lesions with reddish borders and light gray centers with acervuli and Phoma-like species. The presence of these fungi indicates possible pathogenic interactions capable of inciting disease on giant miscanthus. Confirmation of identity through DNA sequencing and pathogenicity of the fungi are being conducted. Further investigation is warranted to better understand the relationship between these fungi and giant miscanthus.

Monitoring sugarcane rust spore concentrations by real-time qPCR and passive spore trapping

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Phytopathology 101:S61

Two rusts are recognized in sugarcane, brown rust caused by Puccinia melanocephala and orange rust caused by P. kuehnii. Both are economically important and limit production in many sugarcane industries. Sugarcane rust epidemics vary greatly in timing and severity, thus they remain difficult to predict. This hampers efficient control using chemical or cultural methods. Real-time qPCR assays for both sugarcane rusts were applied to quantify the number of spores captured on passive spore traps in the 2010 growing seasons in Florida and Louisiana. The limit of detection was found to be a single spore on each trap. Large fluctuations in the number of spores were observed throughout the Florida growing season with spikes evident in June, September and October. These coincided with favorable temperatures and rain events and preceded increases in the severity of orange rust symptoms on susceptible cultivars. The greatest number of orange rust spores detected in Florida occurred in October and coincided with the detection of spores in Louisiana. Symptoms of orange rust have not been reported in Louisiana and this is the first evidence that the pathogen is present in the state and suggests it was transmitted from Florida. These results show the value of combined real-time qPCR and passive spore traps for monitoring temporal and spatial differences in sugarcane rust spore concentrations and provide an important tool for predicting disease epidemics in the future.

Engineering resistance in cotton by RNAi mediated silencing of parasitism genes of Meloidogyne incognita

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Phytopathology 101:S61

In cotton the yield losses due to root-knot nematode are estimated to be in the range of 10–27%. A recent survey in cotton growing areas of north India has revealed widespread infestation of root-knot nematode in Bt cotton. The current battery of candidate nematode specific parasitism proteins secreted by nematodes which can be used for RNAi-mediated nematode management include: Chitinases, cellulases, xylases, expansins, chitinase mutase, proteases, galactouronase, pectate lyase, nodD like and pioneer genes(27) unique to Meloidogyne sp. At CICR Nagpur, work has been initiated on RNAi-mediated protection of cotton against root-knot nematode. Ten sets of primers complementary to the conserved regions of 10 key parasitism genes were synthesized and used for amplification of specific sized amplicons. Evaluation of dsRNA for ten parasitism genes viz. Chitin binding protein, Cysteine protease, Chitin synthase, Integrase, Pectate lyases protein 40, aminopeptidase, polygalactouronase, 16D19, calreticulin was done against root knot nematode penetration. dsRNA for polygalactouronase reduced root knot nematode penetration by 69% compared to control. For pectate lyase the reduction in penetration was in range of 58–74%. However, this reduction in nematode penetration did not translate in proportional reduction in final population build up. Concomitant use of dsRNA of two parasitism genes resulted in significant reduction in penetration as well as final nematode buildup and can be used as potential management option.

Vitis californica and Vitis cf. californica x Vitis vinifera are hosts for Grapevine leafroll-associated virus-2 and -3, and Grapevine virus A and B

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Phytopathology 101:S61

The objective of this research was to determine if native Vitis are alternate Grapevine Leafroll Associated Virus (GLRaV) hosts that might serve as reservoirs important in the continued spread of grapeleaf roll disease. One hundred fifty two Vitis samples surrounding nine Napa Valley vineyards were collected and tested for GLRaV-1 to 5 and -9, Grapevine virus A (GVA), Grapevine virus B (GVB), and Grapevine virus D (GVD) using both conventional and real-time RT-PCR. Twenty four Vitis samples from three riparian areas not near vineyards were also included. DNA fingerprinting indicated that the Vitis samples consisted primarily of V. californica followed by V. californica x V. vinifera hybrids. GVA and/or GLRaV-3 were detected in 53% to 80% of the V. californica and V. californica x V. vinifera hybrids adjacent to three of the nine vineyards. In two riparian areas not near vineyards, three of the 21 F. californica samples were positive for GLRaV-2, GLRaV-3, GVA, and GVB. At the third riparian site, all three V. californica x V. vinifera samples were positive for GLRaV-2 and GVB. Phylogenetic analysis of GLRaV-2 and -3 partial coat protein gene nucleotide sequences indicated the isolates from V. californica and V. californica x V. vinifera hybrids were closely related to V. vinifera isolates. Although we cannot discount the possibility of GLRaV-3 transmission between V. vinifera and native Vitis, we did identify a GLRaV-3 reservoir within a 2 km region of Napa County.
Evidence of root graft transmission of two rose mosaic viruses, Prunus necrotic ring spot virus and Apple mosaic virus in rose rootstocks

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Phytopathology 101:S62

Rose mosaic disease is often caused by Prunus necrotic ring spot virus (PNRSV) and Apple mosaic virus (ApMV). It is primarily spread by propagation; observations indicate a means of natural spread. Cuttings from two rootstocks, *Rosa hybrida* ‘Dr. Huyé’ and *R. multiflora* ‘Burri’, with and without virus were rooted and transplanted to pots. Viruses were: ApMV, PNRSV and a natural infection of ApMV + PNRSV. Treatment pots contained one virus-positive and one virus-negative plant in the same pot to permit root grafting. Control pots contained one virus-positive or one virus-negative plant; pots were arranged to allow stem contact. All virus-negative plants had a possibility of becoming infected by pollen or insect transmission. Dr. Huyé included 60 virus-negative and 60 virus-positive control pots; and 120 treatment pots. *R. multiflora* included 20 virus-negative and 20 virus-positive control pots; and 20 treatment pots with ApMV + PNRSV. All plants were ELISA tested for 5 years. All virus-negative plants in control pots tested negative all 5 years. The percent of initially virus-negative plants in treatment pots that tested positive was 0, 5.3, 10.5, 10.5, 10.5 in Dr. Huyé and 0, 12.5, 33.3, 46.7 and 46.7 in *R. multiflora* for years 1 to 5 respectively. All plants that became infected were potted with ApMV + PNRSV plants. Rose mosaic symptoms were observed only in plants with ApMV + PNRSV. This indicates that root grafting plays a role in spread of rose mosaic disease.

Report of chlorotic ring spot disease on peanuts caused by Tomato yellow fruit ring virus in Iran

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Phytopathology 101:S62

Tomato yellow fruit ring virus (TYFRV; genus *Tospovirus*, family *Bunyaviridae*) is considered as new emerging tospovirus in the mid-Eurasian region of Iran. In the present work, a tospovirus was isolated by mechanical sap transmission from peanut plants showing chlorotic ring spot-symptoms and identified as TYFRV based on biological, serological and molecular studies. In host range studies, a wide range of indicator plants, including members of Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae and Solanaceae, were identified as TYFRV based on biological, serological and molecular studies. In the U.S., QoI resistance was first detected in *P. ipomoeae*. To the best of our knowledge, this study reports for the first time the biological and molecular properties of TYFRV isolates from peanut in the mid-Eurasia of Iran.

Fungal and bacterial diversity differ in their responses to fallow period in the Bolivian highlands

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Phytopathology 101:S62

Traditional fallow periods in the Bolivian highlands are being shortened in an effort to create short-term crop yields with implications for soil communities. Using 454-pyrosequencing, we characterized fungal and bacterial to responses to (1) the length of fallow period and (2) the presence of plants *Parasphaltia sp.* or *Baccharis* sp. (both locally known as ‘Thoła’), locally considered beneficial to soil health. The two study regions, Umala and Ancoraimes, differ in their soil characteristics, which may be a fundamental reason for the inherent differences in regional management practices. Soils in Ancoraimes have higher levels of organic matter, nitrogen and other macro nutrients. These soils supported more diverse fungal communities, whereas Umala had more diverse bacterial communities. Unexpectedly, the longer fallow periods were associated with lower fungal and bacterial richness and diversity. Fungi such as *Bionectria* and *Chaetomium* and bacteria such as *Thermofilum* decreased in abundance with longer fallow period. The presence of *Thoła* after ten years of fallow had a positive effect on soil fungal diversity, but did not change the bacterial diversity. Our results suggest that fallow period has a wide range of effects on microbial communities, and that plant cover may be important in conserving some microbial communities.

Biocontrol potential and plant growth promotion activity of actinomycetes isolated from various herbal vermicomposts

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Phytopathology 101:S62

There is a growing interest in the use of secondary metabolites, such as toxins, proteins, hormones, vitamins, amino acids and antibiotics, from microorganisms, particularly from actinomycetes, for the control of plant pathogens as these are readily degradable, highly specific and less toxic to nature. It is a well-known fact that actinomycetes are found most common in compost and plays an important role not only in the decomposition of organic materials but also in their ability to produce secondary metabolites of commercial interest. Hence, in the present investigation, several herbal vermicomposts were screened for actinomycetes that contain antagonistic potential against *Fusarium* and *isolated* of chickpea (caused by *Fusarium oxysporum* f. sp. *ciceri* [FSC] and *Sclerotium* *rolfsii*, respectively) and charcoal rot of sorghum (caused by *Macrophomina* *phaseolina*). Fourteen most promising antagonistic actinomycetes were characterized for their biocontrol and plant growth promoting traits and further evaluated for their ability to suppress *F. and S. rolfsii* and *M. phaseolina* under both green house and field conditions. The present study was successful in selection effective actinomycetes that can be the potential candidates for discovery of novel secondary metabolites for various biological applications.

Emergence of a plant pathogen via hybridization of the Irish famine pathogen, *Phytophthora infestans*, and an unknown related species

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Phytopathology 101:S62

The global movement of plant pathogens threatens natural ecosystems, food security, and commercial interests. Introduction of a plant pathogen to new geographic regions has been the primary mechanism by which new pathogens have emerged. Another documented mechanism for the emergence of plant pathogens is hybridization between individuals of different species or subspecies. We investigated the genetic origin of *Phytophthora andina*, an emerging pathogen of Andean crops *Solanum betaceum, S. muricatum, S. quitense,* and several *wild* *Solanum* spp. in Colombia, Peru, and Ecuador. We cloned four nuclear loci to obtain haplotypes and using these loci inferred the phylogenetic relationships of *P. andina* to the potato late blight pathogen *P. infestans* and other related species. Sequencing of cloned PCR products revealed two distinct haplotypes for each locus in *P. andina*. Our results indicate that *P. andina* parent and a second divergent allele derived from an unknown species that is closely related but distinct from *P. infestans, P. mirabilis,* and *P. ipomoeae.* To the best of our knowledge, the unknown parent has not yet been collected. We also observed sequence polymorphism among *P. andina* isolates at three of the four loci, many of which segregate between previously described *P. andina* clonal lineages. These results provide strong support that *P. andina* emerged via hybridization between *P. infestans* and another unknown *Phytophthora* species.

Development of a PCR based assay for detection of resistance to QoI fungicides in *Ascochyta rabiei*

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Phytopathology 101:S62

*Ascochyta* blight of chickpea (*Cicer arietinum*) caused by the fungal pathogen *Ascochyta rabiei* is an economically important disease in chickpea. In United States and Canada, management of this disease is dependent on fungicide applications. Azoxytrobin and pyraclostrobin are two quinone-outside inhibitors (QoI) that work by blocking the cytochrome (cyt) bc1 complex (complex III) in the mitochondrial respiration chain and can be used to manage this disease. Resistance to QoI can develop due to single point mutations in the *cyt b* gene. In the U.S., QoI resistance was first detected *A."
Plants wilted and died. Stem and leaf sheath. Symptoms of white mold, but sclerotia and mycelia were detected between the recovered. By day 14, genera. Corms was recorded where appropriate. Disease severity varied greatly among percent plant infected for bunch grasses. Percent of rot in bulbs, rhizomes or was rated as percent stem infection for plants with a single primary stem and inoculated with a mycelial plug of. isolates of. The pathogen often lose significant numbers of plants year after year. Bedding plants resulting in stem rot, wilt and death. Ornamental beds infested. White mold, caused by. Sclerotinia sclerotiorum. E to three varieties per genus were reported susceptibility to white mold. In North Dakota in 2005, and has since been reported in other states. Currently, resistance monitoring of. A. rabiei isolates involves an in-vitro spore germination assay which can be very laborious and time-consuming. Our goal was to identify the mutation associated with Qo1 resistance in this region and to develop a PCR based assay for identification of resistant isolates. Cloning, sequencing and multiple sequence alignment of a fragment of the cytochrome b gene from Qo1 sensitive and resistant isolates of. A. rabiei, revealed that a point mutation in the codon 143 (G143A) was responsible for resistance. A diagnostic test was developed based on this mutation using a mismatch amplification mutation assay (MAMA) with allele-specific reverse primers for screening Qo1 sensitive and resistant isolates of. A. rabiei and is being used for evaluating field isolates.

**Engineering Grapevine fanleaf virus into a plant expression vector**

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Phytopathology 101:S65

Functional genomics studies in grapevine are hindered by the lack of a suitable viral vector for virus-induced gene silencing or protein overexpression. Grapevine fanleaf virus (GFLV) has attributes that could make it a worthy grapevine vector including a small bipartite RNA genome, no plhemo restriction, the availability of functional cDNA clones, and known coat protein residues that abolish nematode-mediated transmission. cDNA fragments corresponding to full-length GFLV RNA1 and RNA2 were cloned into a vector. This construction was transfected into. Nicotiana benthamiana initiated GFLV systemic infection. Reassorting two GFLV isolates (GHu and F13) bipartite genomes has yielded different frequencies of systemic infection and the concomitant application of heterologous viral RNA silencing suppressors has provided new insights into the infection process. Fluorophor-tagged GFLV clones are being tested as a proof of concept for the stable expression of foreign genes in plants. Wild-type and recombinant GFLV are currently being delivered to grapevine.

**Spatial dynamics of Plum pox virus in Prunus spp. in Ontario and Pennsylvania**

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Phytopathology 101:S63

Understanding the spatial dynamics of a disease epidemic can provide important information concerning the development of appropriate sampling designs to optimize detection efficiency. The objective of this project is to quantify the spatial dynamics of Plum pox virus epidemics in Prunus spp. in Ontario, Canada and Pennsylvania. Spatial dependence was measured using a modified form of Ripley’s K function. In Pennsylvania, spatial dependence among PPV-positive Prunus spp. blocks ranged from 0.7 to 4.3 km, whereas in Ontario spatial dependence ranged from 1 to 25 km. Spatial analyses also revealed that PPV-positive blocks were clustered around PPV-positive blocks that had tested positive for PPV the previous year. Within Prunus blocks, PPV-positive trees had a random spatial pattern in most blocks (9 of 12 blocks), while the pattern was clustered in some blocks (3 of 12). Because PPV-positive trees are sometimes clustered within blocks, a systematic sampling design with multiple sampling arms should be used because this sampling design can accommodate both random and clustered spatial patterns.

**Identifying resistance to white mold in annual bedding plants**

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Phytopathology 101:S63

White mold, caused by Sclerotinia sclerotiorum, is a serious disease of annual bedding plants resulting in stem rot, wilt and death. Ornamental beds infected with this pathogen often lose significant numbers of plants year after year. Fourteen genera of annual bedding plants were identified as having no reported susceptibility to white mold. One to three varieties per genus were inoculated with a mycelial plug of S. sclerotiorum, incubated in a dew chamber for 48 hrs, and then maintained at 18C for 4 weeks. Disease severity was rated as percent stem infection for plants with single primary stems and percent plant infected for bunch grapes. Percent of rot in bulbs, rhizomes or corms was recorded where appropriate. Disease severity varied greatly among genera. Portulaca, Pentas, and Scaveola had greater than 50% stem infection by day 14. Impatiens became infected but abcised the diseased stem and recovered. Acorus developed a slow rhizome rot. Penisetum did not exhibit symptoms of white mold, but sclerotia and mycelia were detected between the stem and leaf sheath. Curcium developed soft rot in the corm, and entire plants wilted and died. Colocasia bulbs and Canna rhizomes were free of rot and leaf necrosis was usually restricted to the inoculation site. Juncus, Carex, Cyperus, Setaria and Scirpus developed no disease symptoms. Eight of the fourteen genera were identified as potentially resistant to white mold and will be field tested.

**Global phenotypic variation in Phytophthora capsici**

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To determine global phenotypic variation, 124 P. capsici isolates from 12 regions were characterized for sporangial length and width, pedicel length, oospore diameter, sporangial and chlamydospore production, and growth at 32, 35, and 38°C. Sporangia were 23 to 35 μm wide and 38 to 60 μm long; differences in width and length were noted when isolates were grouped by genetic cluster and continent of origin. Length:breadth ratio (1.34 to 2.07) and pedicel length (20 to 260 μm long) varied widely among isolates; differences were apparent by continent and host family of origin. Oospore diameters varied among isolates (22 to 37 μm), but no differences were noted by isolate genetic cluster, host family of origin, continent of origin, mating type, or sensitivity to mefenoxam. Differences in sporangial production were observed among isolates grouped by continent, and tropical isolates produced fewer sporangia than isolates from vegetable hosts. When cultures were incubated in liquid medium, 35 P. capsici isolates formed chlamydospores. Growth at high temperatures did not reliably separate P. capsici from P. tropicalis in this study. The results of this study indicate that separation of P. capsici from closely related Phytophthora species based on morphological and physiological characters alone could be misleading.

**Differences in virulence of Phytophthora capsici isolates from a global collection**

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Phytopathology 101:S63

Phytophthora capsici causes root, crown, and fruit rot of vegetable and tropical hosts. Cucumber, zucchini, tomato, and pepper fruits were inoculated with 6-mm-diameter agar plugs of P. capsici, incubated in clear plastic boxes at room temperature (~26°C and 100% relative humidity), and virulence was estimated by measuring the lesion diameter three (cucumber, zucchini) or four (tomato, pepper) days later. When isolates were grouped by genetic cluster, differences in virulence were observed for isolates grouped by genetic cluster, but isolates from vegetable crops were generally more virulent than isolates from tropical hosts. No significant differences in lesion diameter were noted on pepper when isolates were grouped by host family of origin or genetic cluster membership. Our findings suggest that isolate characteristics such as host family of origin and genetic cluster membership may be used to guide initial isolate selection for cucurbit fruit resistance screening. Final isolate selection should incorporate the phenotypic and genetic diversity of P. capsici, including isolates with differing virulence to the host organ of interest.

**Exploring the insect vector: virus interactome using co-immunoprecipitation coupled to mass spectrometry**

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Phytopathology 101:S63

Aphid transmission of Potato Leaf Roll Virus (PLRV) requires virions to be internalized into aphid midgut and accessory salivary gland cells, and to survive in the hemolymph suggesting multiple aphid proteins interact with PLRV. We used co-immunoprecipitation to isolate aphid-virus protein complexes, separate by 1-D SDS-PAGE, and then analyze by nanoscale reverse phase chromatography and tandem mass spectrometry. Over 52 aphid proteins were identified with high statistical confidence (P < 0.01) that interact either directly, or in complex, with purified virions with high affinity. Twelve proteins were enriched more than 10-fold and another 12 proteins were specifically co-immunoprecipitated by purified virus. Previously, we reported that some of these 52 proteins were differentially expressed in vector and non-vector species. Using a novel approach in which we identified all aphid proteins reported by others to interact with luteoviruses including actin, GAPDH, and RACK-1, but we did not identify the bacterial endosymbiont protein symbionin (GroEL). Additional proteins identified in this study include virus receptors, cytoskeletal proteins, vesicle trafficking proteins, chaperones, signaling proteins, proteins that modify insect feeding behavior, and enzymes involved in aphid metabolism. These proteins may function at various steps in the circulative transmission pathway to promote virus internalization, translocation in aphid cells, and transmission to new hosts.
De novo generated elf4E resistance genes protect potato from infection by Potato virus Y
Phytopathology 101:S64

Natural mutations in translation initiation factor elf4E confer resistance to potyviruses in many plant species, but no known elf4E-mediated resistance genes are known in potato. We identified a susceptible potato ortholog of an elf4E virus resistance gene from pepper, known to confer resistance to Potato virus Y (PVY), into a de novo allele for resistance to PVY using site-directed mutagenesis. Potatoes were transformed to over-express the mutated potato alleles and tested for resistance to PVY. One of the de novo alleles conferred resistance to all strains of PVY when plants were mechanically inoculated in the greenhouse. Two years of field trials were completed and several lines expressing the de novo allele remained virus free following extreme natural virus inoculum pressure that resulted in >80% of the control plants becoming infected. The resistance was stable through three generations, the plant growth and yield characteristics were similar to untransformed controls, and none of the tubers contained virus. The use of natural or modified elf4E resistance genes to disrupt a key step in the potyvirus infection process could potentially be used to engineer virus resistance in a number of economically important plant-viral pathosystems. Furthermore, the “intrinsic” nature of this approach, whereby the transferred coding region is modified from a gene in the target crop, may be advantageous with respect to consumer acceptance.

The iron responsive sigma factor, AcS, responsible for regulation of aochromobactin biosynthesis in Pseudomonas syringae pv. syringae B728a
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Phytopathology 101:S64

Available iron is extremely limited in many bacterial environments, but is essential for growth and synthesis of many virulence associated factors. Therefore, the study of iron acquisition systems, such as iron-chelating siderophores, is critical to understanding bacterial pathogenesis. The genome of P.s.s. B728a encodes two siderophore systems, the fluorescent siderophore, pyoverdine, and the recently defined citrate siderophore, aochromobactin (ACR). Directly upstream of the 18.4 kb ACR biosynthesis and secretion gene cluster is an extracytoplasmic function (ECF) sigma factor gene, acsS. Genetic and phenotypic analyses were performed using an acsS deletion mutant strain of P.s.s. B728a in low iron conditions, due to the iron responsiveness of this sigma factor. Illumina RNA-Seq analysis of the acsS mutant strain revealed several hundred differentially expressed gene targets, with the ACR biosynthesis gene cluster showing the largest fold change when compared to the wild type B728a strain. Additional studies confirmed that ACR biosynthesis is regulated by the AcS sigma factor. The deletion of acsS also negatively impacted the transcription of numerous flagellar genes and the predicted non-ribosomally synthesized antimetabolite toxin, mangotoxin. Characterization of the regulatory network controlled by AcS will contribute to understanding the ACR siderophore system and the role it plays in the P.s.s. B728a lifecycle.

European nanoviruses: Identification of three new species and new DNA components
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Phytopathology 101:S64

The genome of viruses of the genus Nanovirus (family Nanoviridae) is typically formed by a set of eight circular single-stranded DNAs, each of which is ~1 kb in size and individually encapsidated in unusually small virosp (~18 nm). When characterizing viruses of pea crops in Germany in 2009, we identified a hitherto undescribed nanovirus. Its eight DNA components were sequenced, and clones thereof served to reconstitute an infectious and aphid-transmissible nanovirus. Since this virus differed from known nanoviruses by ~40% in nucleotide sequences we named this new nanovirus the necrotic yellow dwarf virus (PNYDV). Analysis of about 100 symptomatic pea plants collected in Austria, Hungary, Serbia and Sweden in 2010 revealed that >50% of these plants were infected by PNYDVs. Moreover, we identified two further nanovirus species in Europe, which differ in CP amino acid sequences from other nanoviruses by >40%. When sequencing the genomes of these nanoviruses, two strikingly distinct variants of DNA-U2 were encountered in each isolate. In addition to a genetically diverse range of hitherto undescribed para Rep-encoding DNAs (“alphastellitelles”) that we found associated with European nanovirus isolates, we identified a small (503 nts) DNA component as a satellite DNA from 9 of 16 PNYDV isolates from Austria. Our data suggest that nanoviruses are more numerous and widespread in the Old World than originally thought and show new features in genome organization and association with satellite DNAs.

New race of Phytophthora sojae in southern Buenos Aires province (Argentina)
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Phytopathology 101:S64

Phytophthora root rot of soybean caused by Phytophthora sojae is one of the most important diseases in Argentina. Physiological race 1 was identified in the country in 1991 and six years later pathogen variability was detected. Two plants of one supposedly resistant cultivar with typical symptoms of the disease were collected from soybean fields in Tandil (Buenos Aires province). The objective of this study was to report the presence of P. sojae and to determine whether a new race had broken the resistant genes. The isolation was made from the margin of stem lesions, plated on V8 juice medium with antibiotics and fungicides. Cultural characteristics showed identical patterns to those described for P. sojae. The ITS rDNA region was amplified (ITS5/ITS4), sequenced and BLAST aligned with the NCBI and a high similarity with P. sojae strains was verified. In addition, so as to identify the physiological race, the isolate was inoculated by the hypocotyl technique in eight differential soybean isolines: HARO (1-7j) (pprs), HARO 1272 (Rps1a, Rps7), HARO 13 (Rps1b), HARO 14 (Rps1d), HARO 15 (Rps1k), HARO 3272 (Rps3a, Rps7), HARO 6272 (Rps6, Rps7) and Corsoy 79 (Rps1c). Race evaluation was recorded 5 days after inoculation. The virulence/avirulence reaction was: 7,1a, 1c, 1d, 1k and 3a/6b, 1b, corresponding to a new race of the pathogen, capable of defeating Rps1-k and Rps1-c, the major genes used for control of this disease in Argentina. This is the first report of P. sojae in the southern soybean area of Argentina.

Top rot form of red strip caused by Acidovirax avenae subsp. avenae in Louisiana sugarcane
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Phytopathology 101:S64

Red stripe of sugarcane caused by Acidovirax avenae subsp. avenae consists of two forms – leaf stripe and top rot. Symptoms of red stripe in Louisiana over the past 25 years have been limited to the leaf stripe form which causes no apparent yield loss. During 2010, the more severe top rot form was observed in several commercial sugarcane fields. Both forms were found, independently or together. Two fields of cultivar HoCP 00-950, one plant- cane (PC) crop and one first-ratoon (FR) crop, affected by top rot were subdivided into 113 and 84 plots, respectively. In the PC test, plots with >20% affected stalks averaged a 5%, 10%, and 14% loss of tonnes cane/hectare, kg sugar/tonne, and kg sugar/hectare, respectively. In the FR test, the infection level was lower and a 10% loss threshold was utilized, resulting in a 1%, 4%, and 8% loss of tonnes cane/hectare, kg sugar/tonne, and kg sugar/hectare, respectively. A disease incidence, nitrogen fertility rate, and soil texture interaction was noted in plots of nitrogen fertility rate experiment. Incidence was higher among plots in heavy clay soils versus lighter, more silty soils. Disease incidence increased with increasing rates of added nitrogen in the heavy clay soil compared to the control, no nitrogen added plots. In the lighter soil, disease incidence was higher among treatments with added nitrogen compared to the control, but incidence did not differ among the different rates of added nitrogen fertilizer.

The effects of swathing versus straight-cut combining on FHB DON accumulation in barley
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Phytopathology 101:S64

Fusarium head blight (FHB) frequently reduces barley quality, due to the occurrence of deoxynivalenol (DON) mycotoxin. Barley is susceptible to Fusarium graminearum infection and DON formation from head emergence until harvest. The fungus may colonize the barley kernel if dew periods favor fungal growth. Because barley often matures unevenly, it maybe swathed in a windrowed) to accelerate crop maturity and drying. However, barley in a swath may have additional high humidity if rainfall occurs, favoring fungal growth and DON production. To test this, additional mist irrigation was applied after Fekes11.3 to swathed plots and plots left for straight cutting.
over a five year period. Plots were arranged in a split plot design and treatments were applied to Conlon and Robust barley cultivars. The data indicated that for individual years barley type had no significant affect on DON accumulation and DON levels were only significantly different in one year for swathed versus straight-cut plots. Misted plots had significant difference in DON in three out of five years. When years were combined the data indicated swathed and straight-cut plots with added mist irrigation had significantly higher DON than those without mist. barley type and combined type treatments had no affect on DON. Our results indicated that post-dough (Feeke11.3) occurrence of rainfall may have more significant influence on DON accumulation than swathing or straight-cut practice or barley type.

Cytological alterations in Gibberella zeae gerkumps induced by combinatorially selected defense peptides

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Phytopathology 101:S65

Head blight of wheat, caused by the fungus Gibberella zeae, results in reduced grain yield and mycotoxin accumulation. Germlasm with partial resistance to blight is available but efforts are focused on the development of wheat with engineered defense. Previously, we identified peptides from combinatorial phage-display libraries that disrupt ascospor growth and development. If expressed in wheat, these peptides could complement partial resistance. We have initiated studies on the effects of defense peptides on endocytosis, a process cells use for uptake of molecules from the surrounding environment. Endocytosis is central to proper apical growth of fungi. We used the membrane-selective dye, FM4-64, to stain components of the endocytic machinery. We found that germlams derived from ascospores germinated overnight, FM4-64 rapidly stained in succession the plasma membrane, early endosomes, vesicles, and vacuoles. Cells within ascospores and basal hyphae typically contained a single vacuole, comprising most of the cell space, while apical cells contained smaller vacuoles. In germlams grown similarly but in the presence of a defense peptide, FGf3A, FM4-64 staining revealed much smaller and many more vesicles throughout germlams and non-germinated cells of ascospores. Endocytic alterations induced by defense peptides are being compared to the abnormal germlam morphologies that they induce, including isotropic swelling of non-germinated ascospore cells, and smaller and many more vesicles throughout germlams and non-germinated cells of ascospores.

Phytophthora obscura sp. nov. defines a novel Phytophthora subclade 8d

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Phytopathology 101:S65

We describe a new Phytophthora species detected in the U.S.A. infecting foliage of Kalmia latifolia and in substrate underneath Pieris and in Germany in soil samples underneath Aesculus hippocastanum. The species P. obscura sp. nov. is named based on phylogenetic analysis, host range, Koch’s postulates and morphological examination. P. obscura is homothalic with paragamous antheridia and semiapipalate sporangia. P. obscura is genetically closest related to P. adspersa and P. macrochaetae. P. adspersa and P. macrochaetae define a new subclade 8d with significant support for all genetic loci analyzed. Horse chestnut, kalma, pieris and rhododendron could all be infected with this pathogen. Koch’s postulates were confirmed for kalma.

Management of onion bacterial diseases using alternative mulches and plant spacing

Phytopathology 101:S65

Onions are plagued by a number of bacterial pathogens that cause bulb rots and leaf decay. During the past five years, fresh market sweet onion growers in Pennsylvania and New York have lost $3,500 to $7,000 per acre annually as a result of reduced onion quantity and quality due to diseases caused by the center rot pathogens Pantoaea annatans, P. agglomerans, the soft rot pathogens, Pectobacterium carotovora and Pseudomonas marginalis and sour skin caused by Burkholderia cepacia. In on-farm trials, reducing onion plant spacing from the grower standard of 91 cm² (4 rows per bed with 15-cm plant spacing in-row) in PA and 122 cm² in NY, to 81 cm² or 61 cm², in general, reduced the total number of leaves per plant and onion neck diameter, increased percent lodging and reduced bacterial disease incidence at harvest between 52 and 66% when conditions were favorable for disease. However, in some trials this came at the expense of an increased proportion of less marketable small to medium sized bulbs. Growing onions on alternative mulches compared to the grower standard black plastic mulch reduced soil temperatures on average by 1.4 to 5.7°C and reduced bacterial bulb decay between 59 and 75% for the metallic silver, black biodegradable and bare soil/no mulch treatments. Multi-factorial trials combining alternative mulches and plant spacing to reduce bacterial disease losses are planned for 2011.

Inducing of the systemic resistance against Fusarium crown and root rot of tomato (Fusarium oxysporum f. sp. radicis-lycopersici) by rhizobacteria A. Gül (1), H. ÖZAKTAN (2), L. Yolageldi (2), B. Çağr (1)
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Phytopathology 101:S65

Crown and root rot of tomato caused by Fusarium oxysporum f. sp. radicis-lycopersici (FORL) is a common disease in commercial greenhouses in Turkey. The aim of this study is to investigate the effect of rhizobacteria strains isolated from greenhouses on plant growth and biocontrol against FORL by in vivo tests and molecular analysis. As plant material, one resistant (Bandita F.r) and one sensitive (Kardelen F.r) tomato varieties against FORL were used. In the first step, out of 30 rhizobacteria strains were tested for the effects of plant growth-promoting and biocontrol against FORL under in vitro conditions. According to results of in vivo tests, the most effective strains of Pseudomonas, which were effectively inhibited the growth of FORL (TR2/1, Pseudomonas putida) and showed growth-promoting effect (TR2/1, Pseudomonas fluorescens b/v3) were selected for molecular analysis in order to explain their mode of action. For this purpose, we determined ACO1 (LEAC01; regulated by ethylene) gene expression profiles by RT-PCR method. According to the result of molecular tests, TR2/1+FORL application increased the ACO1 gene expression compared to control and only PGPR or FORL inoculated plants. The results of the study showed that PGPR and FORL applications have positive effects on ethylene biosynthesis mediation with ACO1 gene expression and this effect could be important for inducing the systemic resistance of tomato plants against FORL.

Plant and food biosecurity: A European Union Network of Excellence

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Phytopathology 101:S65

A European Union Network of Excellence (NoE) was recently established involving thirteen partner institutions from eight countries: Italy, France, Germany, United Kingdom, Hungary, Turkey, Israel and United States. The consortium will focus on biological threats which have the capacity to infect plants, damage agriculture production and ultimately have impact on food and feed at any stage in the supply chain. Funded by the European Commission’s Programme Security, the NoE includes the following areas of emphasis (Work Packages): Epidemiology and crop biosecurity; Food biosecurity; Analysis of risks to European food systems and society from the intentional introduction of new pest and disease agents; Development and deployment of diagnostic and detection systems; Responder systems for eradication and containment; Training for plant and food biosecurity; Dissemination, awareness and communication. Funded for five years, the Network of Excellence will renew and reinforce a previously established European partnership on crop biosecurity. New countries, institutions and topics have been included with the aim of establishing a virtual Centre of Competence in plant and food biosecurity that will enhance preparedness and response capabilities. The ultimate goal is to develop the capability and capacity to prevent, respond and recover from a biological incident or deliberate criminal act threatening the European agrifood system.

Molecular characterization through IGS sequencing of formae specialis of Fusarium oxysporum pathogenic on lamb’s lettuce and rocket

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Phytopathology 101:S65
Twenty-nine isolates of *Fusarium oxysporum* collected from wilted lamb’s lettuce plants (*Valerianella oltioria*) and thirty-six *Fusarium oxysporum* isolates collected from wilted rocket plants (*Erucica vesicaria L.*, syn. *E. sativa*, cv. ‘Rucola cultivata’), including ATCC strains, were examined for differences in the nucleotide sequences of the ribosomal DNA (rDNA) intergenic spacer (IGS) region, about 2.5 kb long in the isolates analyzed. The isolates were tested for pathogenicity on lamb’s lettuce or rocket in glasshouse. The results showed that the isolates were slightly, moderately and highly pathogenic except for four non-pathogenic isolates from lamb’s lettuce. Most of the isolates from wilted rocket and lamb’s lettuce plants collected from Italy were very similar to *F. oxysporum* f. sp. *raphani*. In conclusion, the analysis of the IGS sequences revealed that the isolates studied had different origins and that phylogeny and pathogenicity were related; non-pathogenic isolates differed genetically from those with low, moderate and high level of virulence. To our knowledge, this is the first report of differentiation of *formae specialis* of *F. oxysporum* on rocket and lamb’s lettuce by IGS sequence analysis.

**Cloning glucanase and chitinase genes from antagonistic yeasts for postharvest disease control**

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Phytopathology 101:S66

Different yeast – including strains of *Pichia guilliermondii*, *Metschnikowia pulcherrima*, and *Metschnikowia fructicola* – have been isolated from the carposphere of fruit, selected for their antagonistic properties against different postharvest pathogens of apples and peaches, and studied for their biocontrol mechanism. *P. guilliermondii* hydrolases is one of the components of the mechanism of action. An exo-1,3-beta-glucanase gene (PgExg1 gene) was amplified from the genomic DNA of the antagonistic yeast *P. guilliermondii* strain M8 and confirmed by Smart-RACE on the cDNA. Sequencing and nucleotide analysis showed that there were no introns inside the gene. An open reading frame (ORF) of 1,224 bp encoding a 408 amino acid protein with a calculated molecular weight of 46.9 kDa was characterized. Protein BLAST revealed that the gene belongs to the cellulose superfamily, and prediction of the deduced amino sequences suggested that the protein has a signal peptide. Similarly, the chitinase genes (*Mpch1* and *MfChi1* genes) were amplified from the genomic DNA of *M. pulcherrima* strain MACHI and *M. fructicola* strain AP47, respectively. Nucleotide analysis showed lack of introns in both. For *Mpch1*, an ORF of 1,080 bp by encoding 359 amino acid protein was characterized, while for *MfChi1*, an ORF of 1,098 bp encoding a 365 amino acid protein was characterized. Protein BLAST revealed that both genes belong to GH18-chitinase-like superfamily.

**Selection of antagonistic yeasts for the control of *Salmonella enterica* serovar *typhimurium* on fresh cut lettuce**

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Phytopathology 101:S66

Fresh-cut products represent a promising and innovative sector, responding to the customer needs and adapting to his lifestyle. Therefore, it is necessary to ensure the microbiological safety of fresh-cut products, because they go through different steps of processing and manipulation (cutting, washing and packing), for this reason they are more prone to contamination than whole products. For this reason *Salmonella* serovar *typhimurium* has been selected as a model pathogen to be isolated from commercial lettuce samples to select yeast strains with antifungal properties.

**Linkage analysis of soybean *Phytophthora* root rot resistance loci on chromosome 13**

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Phytopathology 101:S66

*Phytophthora* root rot, caused by the pathogen *Phytophthora sojae*, is a limiting factor in the production of soybean worldwide. Genes that contribute the expression of *Rps*-mediated and partial resistance toward *P. sojae* provide valuable breeding resources. *Rps3* and *Rps8* are two distinct soybean loci that mediate hypersensitive-response (HR) resistance against *P. sojae*. Both resistance genes have been previously mapped within the resistantrace gene-rich soybean chromosome 13. *Rps3* locus is believed to have three known alleles (*Rps3-a*, *Rps3-b*, and *Rps3-c*) each from a different source; PI 86972-1, PI 82.312N, and PI 340046 respectively. *Rps8* and each of the *Rps3* alleles mediate resistance to different isolates of *P. sojae*. Two *F2* mapping populations were recently generated to elucidate the genetic relationship and segregation pattern between *Rps3* alleles and *Rps8*. The first population originates from a cross between L83-570 (Containing *Rps3-a*) and PI 399073 (Source of *Rps8*), and the second originates from a cross between L92-7857 (Containing *Rps3-c*) and PI 399073. Linkage analysis within chromosome 13 was conducted through combining data from simple-sequence-repeat (SSR) marker polymorphisms, and soybean hypocotyl inoculation tests. Preliminary data from these two populations suggest that this region is segregating for both markers and resistance in a skewed manner, similar to previous studies on these loci.

**The genome of Arachis hypogaea: Genetic linkage map will aid the whole genome sequence assembly**


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Phytopathology 101:S66

The allotetraploid peanut genome assembly will be a valuable resource to researchers studying polyploidy species, in addition to peanut genome evolution and domestication other than facilitating QTL analysis and the tools for marker-assisted breeding. Therefore, a peanut linkage map will aid genome assembly, acting as an independent resource against which contig assembly can be validated. The objective of this study was to develop a comparative integrated map from two recombinant inbred line populations. A total of 4576 SSR markers from three sources: published SSR markers, newly developed SSR markers from ESTs and from BAC end-sequences were used for screening polymorphisms. Two CAP markers were also included to differentiate ahFAD2A alleles and ahFAD2B alleles. A total of 324 markers were anchored on this integrated map covering 1,352.1 cM with 21 linkage groups (LGs). Combining information from duplicated loci between LGs and comparing with published diploid maps, 7 homoeologous groups were defined and 17 LGs (A1 to A10, B1 to B4, B7, B8, and B9) were aligned to corresponding A-subgenome or B-subgenome of diploid progenitors. One reciprocal translocation was confirmed in the tetraploid cultivated peanut genome. Several chromosomal rearrangements were observed. This genetic activity against soil-borne pathogens. A compost originated from green wastes, organic domestic wastes and urban sludges that showed a good suppressive activity in previous trials was used as source of microorganisms. Serial diluted suspensions of compost samples were plated on five different media: selective for *Fusarium* sp., selective for *Trichoderma* sp., selective for oomycetes, potato dextrose agar (PDA) for isolation of fungi, lycogeny broth (LB) for isolation of bacteria. Colonies were isolated from plates and tested under laboratory conditions on tomato seedlings growing on perlite medium in Petri plates infected with *Fusarium oxysporum* f. sp. *radicis-lycopersici* and compared to a commercial antagonist (*Streptomyces griseorividis*, Mycostop, Bioplanet). Among them, those microorganisms showing a biocontrol activity were assessed also under greenhouse condition on three pathosystems: *Fusarium oxysporum* f. sp. basilici/basil, *Phytophthora nicotianae/tobacco* and *Rhizoctonia solani/bean*. None of the microorganisms was able to control the three soil-borne pathogens and only a few to control *R. solani*.
linkage map and others could provide a framework for QTL analysis and a scaffold for integration of the physical map and genome sequence assembly.

Recent advance of plant protection science in China

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Phytopathology 101:S67

Plant protection science in China is a comprehensive discipline involved in the study on biological characteristics of plant diseases, insect pests, weeds and rodents, and their interactions with the environmental factors, and development of IPM theory and technology. Chinese plant protectionists have been focusing on the strategic needs of modern agricultural development, the food security, ecological safety, and income increase of farmers, and carrying on tradition, exploring and innovating, and tackling key problems collaboratively. Through crossover and merger of different disciplines and ceaseless innovation of research technology and measures, the plant protection discipline has achieved the rapid development in research, discipline construction, personnel training, and establishment of scientific research bases. A series of important research achievements and breakthroughs in the branch of plant pathology, agricultural entomology, weed science, rodent control science, biological control, pesticide science, invasion biology and bio-safety supported by the national basic and applied basic research program, high-tech R&D, and applied technology research, have been made during the past five years. A systematic and comprehensive study on mechanisms of important agricultural pest outbreak, and the theory and technology of their prevention and integrated control would be highlighted in the future.

Characterization of the DSF-mediated quorum sensing regulon of Xanthomonas citri ssp. citri

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Phytopathology 101:S67

"Xanthomonas citri" ssp. citri (Xcc) is the causal agent of citrus canker disease. A diffusible signal factor (DSF), characterized as cis-11-methyl-2-dodecenoic acid, serves as a signaling molecule for the communication among Xcc cells. Three genes, *rpfF*, *rpfC* and *rpfG*, are involved in the DSF production, detection and signal transduction. Xcc cells use DSF-mediated quorum sensing to coordinate their gene expression and biofilm dispersal according to the local density of their population. To investigate the role of quorum sensing in citrus canker disease development and characterize quorum sensing regulon, deletion mutants of *rpfF*, *rpfC* and *rpfG* were constructed, and a time-course microarray was applied to analyze the differential gene expression profiles of those mutants and wild type strains in XVM2 medium mimicking the plant extracellular environment. Four hundred and forty-one genes showed differential expression with fold-change greater than 2 in at least one of the three mutants compared to wild type strain. These genes encode proteins and enzymes belonging to 19 functional categories such as production of extracellular enzymes and extracellular polysaccharides, chemotaxis, flagellum biosynthesis, transport and binding proteins. Quantitative real-time PCR is being utilized to further confirm the microarray results.

Effects of grapevine leafroll disease on berry anthocyanins and other flavonoids in a wine grape cultivar

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Phytopathology 101:S67

Red-berried grapevines (*Vitis vinifera*) affected with grapevine leafroll disease (GLRD) produce grapes with uneven ripening and poor color. We conducted molecular and biochemical studies on grapes harvested at different developmental stages from healthy and GLRD-affected (and tested positive for Grapevine leafroll-associated virus 3) wine grape cultivar (cv. Merlot) to gain a better understanding of events leading to these phenotypic changes. Total RNA extracted from berry skin was used in reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) assay, based on SYBR green detection, to study expression of flavonoid biosynthetic pathway genes. A set of reference genes were used for normalization of gene expression data obtained from gene-specific RT-qPCR assays. The overall results showed down-regulation of the pathway genes in berries from GLRD-affected grapevines during véraison and post-véraison stages. Estimation of total anthocyanins, flavonols and proanthocyanidins, and HPLC profiling of different classes of anthocyanins and flavonols further supported the molecular data that metabolism of different classes of flavonoids is altered during the development of berries produced by grapevines infected with GLRD.

Association mapping of stem rot resistance in a world collection of Brassica napus

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Phytopathology 101:S67

Sclerotinia sclerotiorum is a necrotrophic fungus with a broad host range. It is a highly virulent and competitive pathogen. Its resistance spectrum to this pathogen is difficult to identify due to the quantitative nature of resistance and lack of appropriate screening methods. We have developed a reliable stem inoculation protocol for screening of Brassica napus. A world-wide collection of ~400 B. napus accessions were screened by inoculating stems of flowering plants with mycelium of an aggressive isolate (S 321). Disease severity index (DI) consisted of lesion length and depth of stem penetration. DI ranged from resistant (DI = 0) to susceptible (DI = 250). A sub set of 200 lines were selected comprising resistant and susceptible lines from China, Pakistan, Korea, Japan, and Europe. In order to determine population structure within the lines and to relate the SNP data to existing molecular linkage maps, these lines were being screened with microsatellite markers most of which are publicly available. In future they will be used for association mapping using a newly-developed B. napus Illumina GoldenGate SNP array able to query 1,536 loci simultaneously. In addition, eight susceptible and eight resistant lines were selected for gene expression studies using deep RNA-seq data. The objective is to associate transcripts and/or markers with the resistant response and to determine the allelic relationship of QTLs or specific genes among the resistant B. napus lines we have identified so far.

Rapid field-deployable detection of *Ralstonia solanacearum* race 3 biovar 2 in environmental samples using magnetic bead separation and real-time PCR

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*Ralstonia solanacearum* race 3 biovar 2 (R3bv2) is a quarantine pathogen that causes wilting of potato, tomato, geranium and many other crops; Standard real-time PCR (RT-PCR) using R3bv2-specific primers is a sensitive, rapid, and reliable technique that detects as few as 1000 cells ml–1. However, it cannot be directly implemented for detection of R3bv2 in complex environmental samples, such as plant tissues or soil, due to the presence of PCR-inhibitory compounds. Immunomagnetic separation (IMS) and magnetic capture hybridization (MCH) methods were developed to purify and concentrate R. solanacearum cells or DNA free from PCR inhibitors and non-target cells or DNA. These methods utilize paramagnetic beads conjugated to R. solanacearum-specific rabbit antiserum (IMS) or a single-stranded oligonucleotide capture probe that specifically hybridizes to R3bv2 DNA (MCH). After the conjugated beads bind to target cells or DNA, they are magnetically retained while undesired materials are rinsed away. At concentrations as low as ~104 cells ml–1, IMS and MCH increased the sensitivity of subsequent RT-PCR by ~40 and 10 fold, respectively. At lower cell concentrations where direct RT-PCR was not possible, both IMS and MCH allowed detection of R3bv2. Moreover, RT-PCR of plant and soil samples pre-treated by IMS or MCH permitted detection of only R3bv2 strains at ~500 cells ml–1 in ~5 h, whereas detection by direct RT-PCR of these samples was blocked.

Agent-based model of plant virus-host-vector interactions

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In agent-based model, a system comprises of a collection of autonomous decision-making entities called agents. Each entity makes decision based on a pre-defined heuristic rules. In agent-based modeling, the overall behavior of a system emerges out of the interactions of the agents. In this way, agent-based models mimic biological system rather closely. In the last decade, agent-based modeling technique has been used to study epidemiological systems such as smallpox and influenza in human population, yet its utilization in plant disease epidemiology can be assessed. Through this model we hope to show that example, between a plant virus and its vectors host search behavior as it mediates the host. Yet, picturing the epidemiological scale implication of such interactions can be proven daunting. Through this model, the effects of virus infection on individual vector host search, feeding preference, and reproductive variables are simulated and their resultant impact on the virus epidemiology can be assessed. Through this model we hope to show that

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agent-based modeling is a powerful simulation technique available for theoretical study of plant disease epidemiology.

Non-host plant defense against multiple genera of fungal pathogens - initiated with DNase signals released by the pathogen
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Phytopathology 101:S68

Non-host defense resistance is a dynamic, seldom broken, resistance against most of the plant pathogenic fungi. Surprisingly, the fungus itself is a source of DNase signals that can activate the plant's defense. When not sufficiently activated, a plant can be susceptible to specific pathogens. The cloning of fungal DNase reveals that N-terminal amino acid sequences possess signal peptides that enable secretion, entrance to the pea plant cell and the single stranded viral RNA, during this active period. Proposedly, this action promotes the transcription of genes in sensitive regions of the chromosome. DNases have not been previously touted as major signals. We recently reported the differential depletion of histone H2A and HMG A protein from the DNA sequence in the vicinity of a PR gene (DRR206) 4 h after inoculation with Fusarium solani f. sp. phaseoli (Fsp) or psi (Fspi) (Plant Sci. 177:439). Currently, we discovered defense-inducing DNases from several major genera of plant pathogenic fungi: Puccinia, Colletotrichum, Verticillium, Phytophthora, and Rhizoctonia. The DNase genes from Fusarium solani and Verticillium dahliae have been cloned and specific cons. of the purified enzyme used both to induce resistance in pea to Fspi and to break resistance against Fsp.

Production practices and cultivar selection impacts the occurrence of diseases and the yield of peanut
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Phytopathology 101:S68

In 2009 and 2010, impact of tillage, planting date, cultivar, and row pattern on the occurrence of tomato spotted wilt virus (TSWV), leaf spot, and stem rot along with peanut yield was evaluated. Plot design, with four replications, had conventional or row tillage as the whole plot with late April, mid-May, and early June planting dates as the split plot, Georgia Green and Tifguard cultivars as the split-split plot, and single or twin row patterns as the split-split-split plot, which had four 30-ft rows. While row pattern had little impact on disease, significant 2- or 3-way interactions between tillage, planting date, and cultivar on the occurrence of TSWV, leaf spot, stem rot, and yield were noted. Overall, Tifguard often had lower disease ratings and higher yield than Georgia Green. Under conventional tillage, TSWV, leaf spot and stem rot ratings trended higher on Georgia Green than Tifguard. While stem rot incidence was often highest with the mid-April planting date, higher leaf spot ratings were seen with the early June planting date. Impact of tillage on leaf spot and stem rot varied by year. Higher yields were obtained with the twin compared with single row peanuts in 2009, while significant yield gains with the twin row pattern were noted for conventional but not row tilled peanuts in 2010. Results suggest that lowest disease and highest yields would be obtained by planting Tifguard on twin rows using conventional tillage.

Impact of nitrogen rate and variety selection on disease severity and yield of rainfed forage and sweet sorghum grown for biofuel
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Phytopathology 101:S68

Impact of nitrogen rate on the biomass and sugar yield, as well as disease severity on selected sweet and forage sorghum varieties was assessed in 2009 and 2010 on a site in continuous sorghum production in southwest Alabama. Study design was a split plot with nitrogen rates of 20, 40, 60, and 80 lb actual N/ha as the main plot and sorghum variety, which in both years included Dale and M81-E sweet sorghum and SS1515 forage sorghum, as the subplot treatments. The sweet sorghum Sugar Drip was replaced with the forage sorghum SS405 in 2010. The study was not irrigated. No nitrogen × sorghum variety interaction for any yield or disease variable were noted. Variety selection had a significant impact in both years on fresh (wt), dry, and bagasse yield as well as brix values and sugar yield, and overall disease severity. While variety selection also significantly impacted sugar cane borer (Diatraea saccharalis) damage in 2009, insect activity was minimal in 2010. Primary disease was anthracnose (Colletotrichum graminicola) on M81-E and SS405, and rough leaf spot (Ascochyta sorghi) and/or zonate leaf spot (Gloeosporiopsis sorghi) on Sugar Drip, Dale, and SS1515. While M81-E had the highest biomass yield in 2009, that variety and SS405 had equally high biomass yields in 2010. Highest brix values and sugar yield were obtained in both years with M81-E and Dale. Disease severity was not impacted by nitrogen rate. Equally high biomass and sugar yields were obtained across all nitrogen rates.

Drench and foliar fungicides compared for control of Entomosporium leaf spot on photinia
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Phytopathology 101:S68

Efficacy of the soil fungicide drench All-In-One Rose and Flower Care Concentrate was compared with the foliar fungicides Daconil Weather Stik 6M, Disease Control Concentrate, Disease Covers & Shrubs Concentrate and RosePride Disease Control Concentrate for the control of Entomosporium leaf spot on field-grown red-tip photinia (Photinia x fraseri 'Birmingham'). While the All-In-One drench was poured over the root zone monthly from January 4 to July 5, 2006; January 12 to July 11, 2007; and January 17 to June 23, 2008, the above foliar fungicides were applied at standard rates during this study period. Disease severity was periodically rated using the 1 to 10 Florida peanut leaf spot scoring system of during the winter and spring of each study year. When Entomosporium leaf spot data were pooled over years, All-In-One drench and the non-fungicide treated-photinia had similarly high AUDPC values, in contrast to the significantly lower values recorded for all the foliar fungicides. In May, defoliation levels on the non-fungicide treated controls, which ranged from 25 to nearly 75% in 2006 and 2007, respectively, reached similar levels in all 3-years on the All-In-One-treated plants. In contrast, little leaf spotting and no defoliation was seen on the photinia treated with Daconil Weather Stik 6M, Immunox, Disease Control and RosePride. Poor Entomosporium leaf spot efficacy of the All-In-One was attributed to an inadequate rate of the tebuconazole component.

Pathogenicity test of four potential fungal biocontrol agents on Setose Cephalanoplos weed and their safety on agricultural crops
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Setose Cephalanoplos weed, Cephalanoplos setosum (willd) Kitam, is a economically significant weed in the fields. To develop biocontrol agents as alternative management method to herbicide, the pathogenicity to C. setosum (willd) Kitam was tested by four fungal pathogens Sclerotinia sclerotiorum, Alternaria sp., Epicoccum nigrum and Fusarium tricinctum which were isolated from diseased plant of C. setosum (willd) Kitam. The results of plant virulence assay indicated that S. sclerotiorum is a highly virulent pathogen that could cause foliar and stem necrosis. Alternaria sp. and E. nigrum could cause symptoms on the leaves of C. setosum (willd) Kitam, However, F. tricinctum only showed a low level of virulence among the weed. The pathogenicity test of different combination among the four pathogens to C. setosum (willd) Kitam was also conducted, the mixture of 3 pathogens (S. sclerotiorum+ Alternaria sp.+ E. nigrum) in 1:1:1 ratio showed higher virulence than individual inoculums. All the leaves blighted in 5 days after inoculation, and the whole plants died after 10 days. The safety of these potential biocontrol agents to major crops in Qinghai was tested by bioassay. The test showed that combination of 3 pathogens were virulent to wheat and highland barley, but safe to broad bean and pea. The results suggested that the combination of 3 strains have a high potential to be developed as fungal herbicides to the weeds C. setosum (willd) Kitam in the fields of broad bean and pea.

Expression of hemolysin (exotoxin) of 'Candidatus Liberibacter asiaticus' in citrus using Citrus tristeza virus vector
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Citrus Huanglongbing (HLB) also known as citrus greening is one of the most destructive diseases of citrus worldwide. The causative agent Candidatus Liberibacter asiaticus (CLas) is a fastidious, Gram-negative, phloem-limited, alpha-proteobacterium. To understand how CLas induce disease in citrus, it is important to express the CLas effectors directly inside the phloem. Using bioinformatics tools, we have identified a number of putative effector genes based on the genome sequence of CLas. One of the important effectors or virulence factors is hemolysin, a 50 kDa protein secreted by type I secretion system. The hypothesis is that the hemolysin might interfere with metal ion transportation leading to host metabolic imbalance potentially resulting in disease symptoms. By the use of citrus tristeza virus (CTV) vector, we could express hemolysin effectors of the CLas bacterium directly inside the phloem of citrus. Hemolysin gene from CLas was amplified, engineered into a binary vector based CTV vector and agro-inoculated to Nicotiana benthamiana seedlings. CTV virions containing hemolysin effector were purified and inoculated to citrus plants by bark-flap inoculation. The resulting systemic
spread and expression of the putative effectors throughout citrus trees will enable us to understand the role of the putative effector in disease induction.

**Study of Citrus exocortis viroid replication in citrus protoplasts**

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Phytopathology 101:S69

*Citrus exocortis viroid* (CEVd) is single-stranded, circular, non-encapsidated, non-coding RNA that infects citrus and other hosts. CEVd replicates in the cell nucleus using host polymerase. Citrus protoplast systems coupled with a quantitative (q) PCR assay was developed to study and monitor viroid replication. A viroid target was placed at the cellular level. We were able to obtain up to 40 million protoplasts reliably from a 40 ml of suspension culture of *Citrus amblycarpa*. The level of CEVd progeny RNA increased by 2.5 fold at 2 days post-transfection (dpt) compared to 1 dpt. The level of accumulation of progeny CEVd RNA increased to 80, 100 and 130 fold by 3, 4, and 5 dpt, respectively. Asymmetry in the ratio of the replicative intermediates and progenon molecules was also observed (analogous to the negative-strand in positive-stranded RNA viruses). The ratio of positive-strand to negative-strand varied with time of incubation and peaked at 3 dpt, with a ratio of 15:1. The protoplast system developed in this present study should allow in-depth investigation of the structural elements of CEVd essential for replication, as well as potential host factors for replication.

**Presence of the potato late blight resistance gene Rpi-blh1 does not promote adaptive parasitism of Phytophthora infestans**

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Phytopathology 101:S69

The gene Rpi-blh1, from the wild potato species *S. bulbocastanum*, confers partial resistance to late blight, caused by the oomycete pathogen *Phytophthora infestans*. In order to determine whether a single strain of *P. infestans* can adapt to overcome this partial resistance source, we subjected Rpi-blh1 containing plants to multiple rounds of infection with *P. infestans*, with sporangia from a late blight lesion used to infect the next leaflet. A parallel line of inoculations was done using susceptible leaflets. At the end of the experiment, sporangia passed through resistant or susceptible leaflets were compared for their ability to cause disease. Variants of the corresponding *P. infestans* effector IPi-O, which is recognized by the Rpi-blh1 protein to elicit resistance, were also cloned and sequenced to determine whether variation occurred after selection on the partially resistant host. After 20 rounds of selection, no breakdown in Rpi-blh1 resistance was observed. In fact, the strain that was continually passed through the partially resistant host produced smaller lesions on susceptible leaflets and had a lower infection frequency than the strain passed through susceptible cultivar Kaithadun. Our results indicate that continual exposure to the Rpi-blh1 gene can reduce *P. infestans* virulence.

**Effect of temperature on survival of Phytophthora and bacterial species in irrigation water**

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Phytopathology 101:S69

Plant pathogens, especially *Phytophthora* species, in re-circulating irrigation system present a significant health risk to floral crops. One of current technologies for water disinfection is heat treatment, which is highly effective and has minimal human health and environmental hazards. The primary objective of this study was to examine whether water temperature required to inactivate major pathogens in re-circulated irrigation water can be lowered from 95°C, the recommended temperature in the current protocols. Specifically, we investigated the effect of water temperature on zoospore survival and infectivity of *P. nicotianae* on annual vinca (*Catharanthus roseus* cv. Little Bright Eye) under greenhouse conditions, and on survival of six common plant and one human bacterial species. Inoculation of annual vinca with zoospore suspensions of *P. nicotianae* treated at 42°C for 12 h or at 48°C for 6 h did not result in any disease. None of the seven bacterial species survived 48°C for 24 h. Comparatively, *P. syringae* was the most heat-sensitive, while *E. coli*, *C. carotovora*, and *R. solanacearum* were more heat-resistant. In conclusion, along our previous work on oospores and chlamydospores of *Phytophthora* spp., the results suggest that required water temperature for heat treatment may be lowered substantially from 95°C without sacrificing efficacy. This research is being expanded to understand the underlying mechanisms of pathogen killing at lower than physically lethal temperatures.

**Fungidal efficacy of oxyxsilver nitrate and sodium dipoitdagoargentate (III) for control of seed-borne and foliar diseases**

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Phytopathology 101:S69

Silver and oxidized silver compounds have recently been shown to be effective plant disease management tools. For example, some non-oxidized silver compounds are effective fungicides when applied to inoculated foliage. Oxidized forms of silver (e.g. oxyxsilver nitrate) also have significant potential in crop protection, particularly as seed treatments for eradicating seed-borne bacteria and fungi on pulses and other crops. We report here that oxidized silver compounds, oxyxsilver nitrate and dipoitdagoargentate (III), are potential disease management tools for field and horticultural crops as seed treatments and foliar-applied fungicides. In replicated, small-plot field trials, oxyxsilver nitrate was shown to reduce seed piece decay (*Fusarium sambu- cinum*) on potato. Furthermore, both oxyxsilver nitrate and sodium dipoitdagoargentate (III) reduced foliar diseases on field crops including Ascochyta blight (Ascochyta rabiei) on chickpea and white mould (*Sclerotinia sclero- tiorum*) on dry bean. The ability of oxidized silver compounds to reduce disease symptoms was especially evident when tank mixed with other commercially available fungicides such as Bravo®500® and Allegro®500®. In tank-mixed treatments enhanced, and perhaps synergistic, efficacy was observed.

**In vitro sensitivity of the Pythium blight pathogens of snap bean to various fungicides**

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Phytopathology 101:S69

Sensitivity of the pathogens causing Pythium blight on snap bean was determined in *vitro* against the active ingredients azoxystrobin, cyazofamid, mefenoxam, copper hydroxide, and potassium phosphate, which are fungicides commonly used in snap bean production. Twenty-two *Pythium* isolates were collected from symptomatic plants in Virginia, Georgia, and New Jersey, representing *P. aphanidermatum*, *P. deliense*, *P. ultimum*, and *P. myriotylum*. Isolates were placed on media amended with each active ingredient at 0, 100 µg/ml, the concentration equivalent to the labeled rate if applied on succulent beans at 187 L/ha, and the equivalent if applied at 374 L/ha. All isolates were completely sensitive (100% growth reduction, or GR) to all active ingredients at the labeled rates, except azoxystrobin. GR due to azoxystrobin showed a wide range across the isolates. At 100 µg/ml azoxystrobin, one *P. deliense* isolate demonstrated 8.9% GR. All isolates had 100% GR to copper hydroxide at 100 µg/ml, and the lowest GR on mefenoxam-amended medium was 91.9%. *P. aphanidermatum* isolates varied in sensitivity at 100 µg/ml cyazofamid, ranging from 69.2 to 100%, and all *P. deliense* isolates were completely sensitive. At 100 µg/ml potassium phosphate, significant GR similarities were recorded within isolates of the same species, and less than 50% GR was observed in all *P. deliense* isolates. No relationship was observed between collection location and isolate sensitivity.

**Evaluation of Raspberry (Rubus sp.) cultivars for postharvest quality and resistance to Botrytis cinerea**

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Phytopathology 101:S69

Raspberries (*Rubus* sp.) are a delicate high value specialty crop with an extremely short shelf life. This is exacerbated by their susceptibility to postharvest decay caused by *Botrytis cinerea*. Of the three commercially available available species, red raspberry (*Rubus idaeus* subsp. *Strigosus*) is the most widely grown. Yellow (*R. idaeus*), black (*R. occidentalis L.*) and purple raspberries (*R. neglectus Peck, or R. occidentalis × R. idaeus hybrids*), are mainly available at local markets and U-pick farms. There are no recent studies examining post-harvest quality differences between these raspberry types and the role of host genotype in decay resistance. Therefore, the post-harvest quality of 13 cultivars of red, yellow, purple and black raspberries was examined twice weekly from June to September in 2010. Storage life was assessed at 4°C and 20°C for 6 days by recording decay incidence and percentage bleed. Firmness, color, respiration and ethylene emission rates were measured in select harvests. Additional raspberries were inoculated with 3 different B. cinerea isolates to examine their levels of decay resistance. Preliminary results show that black and purple raspberries outperformed the red and yellow varieties in storage. Black raspberries had the lowest ethylene emission rates and incidence of decay when inoculated with B. cinerea. Future studies will focus on confirmation of 2010 decay data and the relationship between B. cinerea resistance and cultivar physiology.
Diversity of TonB-dependent outer-membrane proteins in plant-associated strains of *Pseudomonas fluorescens*

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Phytopathology 101:S70

Genomic sequences of ten strains of plant-associated *Pseudomonas* spp. were surveyed for the presence of TonB-dependent outer-membrane proteins (TBDPs), which function in the uptake of substrates from the environment by many Gram-negative bacteria. The ten strains represent *P. fluorescens*, *P. chlororaphis*, and *P. synxantha* isolated from the phyllosphere, rhizosphere or soil. 14 to 45 TBDPs were identified in each strain, and phylogenetic analysis of the TBDPs identified five that are conserved across all ten genomes. Comparisons to proteins with known functions allowed the assignment of putative roles in uptake of heme, vitamin B12, copper, and the siderophore ferrichrome to the conserved TBDPs. Each strain also has multiple TBDPs with predicted functions in the uptake of pyoverdines, a structurally diverse class of siderophores produced by the fluorescent pseudomonads. For example, strain Pf-5 has six such TBDPs. Using crossfeeding assays, we found that Pf-5 utilized pyoverdines having 17 distinct structures. Mutants of Pf-5 lacking each of the six putative pyoverdine receptors were constructed and tested in crossfeeding assays, which linked the uptake of specific pyoverdines to individual TBDPs. The identification of the core TBDPs present in all genomes as well as the TBDPs unique to each genome highlights functions conserved across the species as well as those specific to the distinctive lifestyles of each strain.

**Evolution of the ‘Ca. Liberibacter asiaticus’ genome for and intracellular lifestyle**

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Phytopathology 101:S70

‘Ca. Liberibacter asiaticus’ (CaLas) is a member of the Rhizobiiales. Intracellular pathogens, Calas and *B. henselae* have genomes with many characteristics reduced genomes adapted to their lifestyle. This includes drastically reduced gene content and genome size as well as a much lower content of guanine and cytosine. Codon and amino acid preferences that emphasize low guanosine and cytosine usage are employed globally in these genomes. The length of orthologous proteins is generally conserved, but not their isoelectric points, consistent with extensive amino acid substitutions to accommodate selection for a low GC genome. Massive amino acid substitution requires an equally massive accumulation of mutations to the genome. This remarkable process could be facilitated by impaired DNA replication and repair capabilities. Detailed analysis of the repertoires of enzymes required for DNA replication and repair in *CaLas* and *S. melliloti* suggests that the ability of CaLas to repair mutations in its genome may be impaired. The AT-rich genome of intracellular pathogens is likely selected for by energy savings for both the pathogen and the host. This includes a lower metabolic cost for AT vs GC base pairs. We hypothesize that replication and transcription of an A+T-rich genome is energetically favored by a lower ATP cost for strand separation by DNA helicase. The reduced genome of CaLas enables an expanded host range, including diverse plant hosts and the citrus psyllid.

**Determining the prevalence and distribution of bacterial diseases in Nebraska dry bean production fields**

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Phytopathology 101:S70

Dry beans in Nebraska may be affected by a complex of different bacterial diseases, including common blight, halo blight, brown spot, and wilt. Control measures are more readily available for some of these diseases than others, therefore correct identification of the various bacterial diseases and determining their incidence and distribution across the different growing regions of Nebraska becomes important for choosing between the various options for disease management. A comprehensive survey was conducted between early June and mid-September, over two seasons (2009–2010) to establish the presence of the most important diseases and whether any are predominating in some production areas but not others. The survey included 519 samples from multiple market classes of dry beans representing 222 fields from 11 counties in western Nebraska. Wilt was found to be the most commonly occurring disease (23.5%). The other three diseases were found readily in fields: halo blight (9.5%), common blight (17.5%), but the presence of brown spot (15.5%) was much higher than expected over the two year project. Based on the results of this study, resistance evaluations are currently being conducted for wilt and brown spot in the effort to produce new highly resistant cultivars for use in Nebraska.

**Genetic and biological variability of Pepino mosaic virus isolates infecting tomato plants**

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Phytopathology 101:S70

Pepino mosaic virus (PepMV) is considered as one of the most dangerous pathogens infecting tomato worldwide. The virus is highly diverse; four distinct genotypes have been described so far. The aim of our work was to determine relation between different PepMV isolates and the severity of symptoms expression in infected tomato plants. Several Polish isolates, (Chilean 2 genotype-CH2) displaying wide range of symptoms from mild mosaic to severe necrosis on tomato plants, were used in this study. The coat protein, triple gene block and part of polymerase genes were amplified using PepMV specific primers, cloned and sequenced. The sequences comparison were performed and single nucleotide polymorphism sites were identified. Analysis of symptoms and their correlation with specific amino acid positions was also performed. Sequence comparison showed up to 99% identity between CH2-mild and CH2-necrotic isolates. Mutations affected on amino acid changes were randomly distributed however unique nucleotide substitutions in isolates causing leaf necrosis and yellowing were observed. Results of this study show that different symptoms induced by PepMV isolates on tomato plants may be related to minor differences at the nucleotide level between them. These results might help in future identification of genome regions involved in the expression of PepMV symptoms in tomato.

**Characterization of a novel Emaravirus infecting blackberry**

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Phytopathology 101:S70

Several new viruses have been identified in blackberry in the last decade. These viruses are usually found in mixed infections and are associated with blackberry yellow vein disease (BYVD), a disorder caused by virus complexes. In order to investigate the epidemiology of the viruses involved in the disease, virus-tested sentinel blackberry plants were placed in a field with high BYVD incidence and several developed viral-like symptoms after only a month exposure in the field. Symptoms included leaf mottling, chlorotic ringspots and curved midribs. Some of the symptomatic plants were subjected to deep sequencing using the Illumina platform and regions of an apparently new virus were obtained. The genome organization of the new virus is similar to that of emaraviruses and phylogenetic analysis showed that it is closely related to Fig mosaic and Rose rosette viruses. RT-PCR detection protocols have been developed and successfully used to detect the virus in BYVD plants. These tests showed that the new virus is prevalent in blackberry fields across with the etiology of BYVD. Mite transmission experiments are being conducted to identify the vector that accounts for the rapid movement of the virus.

**Editing in Wikipedia to learn concepts in plant pathology**

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Phytopathology 101:S70

Wikipedia is a collaboratively written online encyclopedia. Wide use by the public and the fact that anyone can edit its articles makes it an accessible format for students to share information they have learned. This study examines the value of editing in Wikipedia as an assignment for a college plant pathology course. It was hypothesized that the assignment would generate student enthusiasm and therefore lead to better learning outcomes and a higher quality product than a standard written paper. Success was evaluated with a student survey and an instructor evaluation of the final product. We found that student enthusiasm was indeed a positive outcome of this assignment and that we did generally achieve our learning goals. However, we did not find that the quality of the final products were any better. We think this is valuable assignment and we will continue to use it with a few revisions aimed at increasing the connection to lecture concepts and an emphasis on writing clarity and use of citations.

**Accessing phosphoglucone isomerases: A gene with potential links to fitness and invasibility of the leafroller *Epiphyas postvittana* (Lepidoptera)**

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Phytopathology 101:S70
Light brown apple moth, *Epiphyas postvittana*, is a significant horticultural pest native to Australia, and currently with a limited global distribution. However, it can tolerate very heterogeneous climatic and vegetation conditions and has recently invaded California with considerable consequences for U.S. international and domestic trade. A genetic factor that may contribute to its environmental adaptability, and consequently invasive capability, is the phosphatase gene *pgi* (*phosphoglucomutase* gene). This gene codes for a key enzyme in the second step of glycolysis and for which the isozyme composition has been associated with the fitness and dispersal capacity of other moths. As a first step, to determine if this locus is variable within *E. postvittana*, novel primers were designed enabling access to 957 bp of the coding region across exons 4 to 11 of *pgi*. Exon-primed intron-crossing (EPIC) primers were then designed to compare sequences of 17 species across one laboratory and three wild New Zealand populations from a latitudinal range of ~39–45°S. A total of 70 segregating sites in the exons were found, including 61 synonymous and nine nonsynonymous. Introns 3 to 11 (excluding intron 10) were also sequenced for 13 individuals revealing significant length variation within and between introns and populations. The level of variation revealed here indicates that this could be a useful target gene to assess fitness factors associated with invasibility of *E. postvittana*.

### Transgenic rice with inducible overproduction of ethylene exhibits broad-spectrum disease resistance

#### Transgenic rice with inducible overproduction of ethylene exhibits broad-spectrum disease resistance

E. E. HELLIWELL (1), Q. Wang (2), Y. Yang (2)

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**Sporulation dynamics of Spilocaea oleaginea and timing of olive leaf spot infection in the orchard**

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**Phytopathology** 101:S71

Fall infections of olive leaf spot, incited by *Spilocaea oleaginea*, have a major impact in defoliation of olive trees. Control measures are based upon the spray of fungicides starting with the first fall rains. Sporulation dynamics were studied washing 20 individual lesions every 2 weeks since October 2008 to May 2010, in 2 Chilean orchards located in the coastal and central regions. Sporulation was expressed as the number of conidia in one centimeter of spot. Conidia were produced continuously in the coastal orchard with the highest amounts produced by mid springs and lowest amounts during the summers. A discontinuous production was observed in the central orchard with initial high sporulation in the spring 2008 that ended in early summer due to defoliation, reasuming at the end of winter 2009. Timing of fall infections was studied in 3 orchards where individual branches were covered with paper bags, to avoid infections. 50 branches were unwrapped at monthly intervals from April 22th until October 28th 2010, allowing 7 exposition periods for infections to occur. Infections were found in all exposition periods with higher incidence during the months of April, May and June. Differences among orchards were related to the different climatic conditions, with higher incidence in the coastal orchard. These results indicated that disease onset started before the first rains and are the result of free water coming mainly from morning dew. Therefore, fungicides should be sprayed earlier.

### Inhibitory effects of Bacillus amylolyticus and Paenibacillus polymyxa on Botrytis cinerea causing gray rot of grapes

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**Phytopathology** 101:S71

A collection of seven strains of bacteria isolated from leaves of table grapes and an apple fruit were screened *in vitro* for inhibition of *Botrytis cinerea*. For the *in vitro* inhibition assay, the bacteria were directly transferred from a pure culture 0, 24, 48 and 72 hours before pathogen inoculation on potato dextrose agar (PDA) media, using a 5 mm *B. cinerea* PDA plug. Plates were incubated at 17 ± 2°C for 7 days. Two of the strains obtained by washing 20 individual lesions every 2 weeks since October 2008 to May 2010, in 2 Chilean orchards located in the central and coastal regions. Sporulation was expressed as the number of conidia in one centimeter of spot. Conidia were produced continuously in the coastal orchard with the highest amounts produced by mid springs and lowest amounts during the summers. A discontinuous production was observed in the central orchard with initial high sporulation in the spring 2008 that ended in early summer due to defoliation, reasuming at the end of winter 2009. Timing of fall infections was studied in 3 orchards where individual branches were covered with paper bags, to avoid infections. 50 branches were unwrapped at monthly intervals from April 22th until October 28th 2010, allowing 7 exposition periods for infections to occur. Infections were found in all exposition periods with higher incidence during the months of April, May and June. Differences among orchards were related to the different climatic conditions, with higher incidence in the coastal orchard. These results indicated that disease onset started before the first rains and are the result of free water coming mainly from morning dew. Therefore, fungicides should be sprayed earlier.

#### Inhibitory effects of Bacillus amylolyticus and Paenibacillus polymyxa on Botrytis cinerea causing gray rot of grapes

B. amylovorus, B. amylolyticus, and P. polymyxa are Gram-positive bacteria that produce amylase and exoproteinase enzymes which break down starch and cellulose, respectively. These enzymes play a role in the pathogenesis of gray rot disease in grapevines. For this study, seven strains of bacteria were selected based on their ability to inhibit the growth of *B. cinerea* in *in vitro* assays. The strains were characterized using conventional methods, including antimicrobial susceptibility testing, carbohydrate utilization, and growth on a variety of media. The results showed that all seven strains were able to inhibit the growth of *B. cinerea* at different levels, with *B. amylolyticus* and *P. polymyxa* showing the highest inhibitory activity. The inhibitory effect of these bacteria on *B. cinerea* was further evaluated using a plant infection assay. The results showed that the bacterial strains were able to significantly reduce the severity of gray rot disease in grapevines.

### Functional biodiversity: Study of the raspberry bush - Rubus idaeus (rosaceae)

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**Phytopathology** 101:S71

Natural ecosystems are usually self-balancing. Interactions between plants and animals are regulated by various mechanisms, without the need for human intervention. In protected raspberry culture, we have researched many cases of failure of integrated pest management. The analysis of causes for these failures was found to be extremely complex, due to the numerous interactions between organisms. The aim of this work is to offer a simplified assessment scheme for the interactions present across physiologioal and entomological data. We observed that: 8 direct auxiliairies of *R. idaeus* are also
present on associated plants; The enormous biomass, mainly made up of 217
neutral arthropods for R. idaeus, is composed of indirect auxiliaries because
they act as additional food to the 8 direct auxiliaries; 13 associated plants contribute to maintaining the auxiliary populations.

Influence of Maize mosaic virus on the fitness and wing morphology of
Peregrinus maidis (Hemiptera: Delphacidae)
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Phytopathology 101:S72

Maize mosaic virus (MMV; Rhabdoviridae) is a corn virus transmitted
propagatively by the corn planthopper, Peregrinus maidis (Hemiptera: Delphacidae). We examined the fitness of P. maidis developing on MMV-infected or healthy corn leaves; however, our results showed little difference in mean developmental time, mean fecundity, and mean longevity. Delphacid planthoppers can differentiate wing dimorphic forms called either macropters (long wing forms) or brachypters (short wing forms). Since the abundance of these forms may vary in response to the physiological status of the host plant, we examined the effect of MMV on the wing morphology of P. maidis. Our results showed that planthoppers that developed on young (21–28 days old) infected plants produced 17% more of brachypters than planthoppers that developed on healthy plants of the same age. Conversely, planthoppers that developed on old (42–49 days old) infected plants produced 16% more of macropters than planthoppers that developed on healthy plants of the same age. Our results suggest that MMV infection may regulate the density and dispersal of planthoppers according to the stage of plant infection. At early stage, the virus may increase the vector population by producing more brachypters; however, at later stages of plant infection the virus may promote vector dispersal by triggering larger production of macropters.

Nuclear magnetic resonance for non-destructive imaging of belowground
damage caused by Heterodera schachtii and Rhizoctonia solani on sugar
beet
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Phytopathology 101:S72

Belowground symptoms of sugar beet caused by the beet cyst nematode (BCN) Heterodera schachtii include the development of compensatory secondary roots and beet deformity that can be assessed only following destructive removal of entire root systems from the soil. Infections by the soil-borne basidiomycete Rhizoctonia solani cause brown or black decay on beet and roots (Rhizoctonia crown and root rot, RCRR). Nuclear magnetic resonance imaging was applied for the detection of belowground symptoms caused by BCN and/or RCRR on sugar beet. Excessive lateral root development and beet deformation of plants infected by BCN was obvious 28 days after inoculation (dai) on resonance images when compared to non-infected plants. Three dimensional resonance images recorded 56 dai gave insight on BCN cyst development. Taxonomic studies are underway to determine the exact relationship of this pathogen within the guava rust complex. It has now been identified on 55 species of Myrtaceae but further seedling testing suggest that most Myrtaceae might be susceptible to the disease although there are some indications of useful sources of host plant resistance. Other studies focus on modelling environmental impact based on current data. We are seeking science collaborations to broaden our understanding of the ecology and behavior of this disease relative to other members of the guava rust complex.

Effect of soil-incorporated cover crops and Actinovate biocontrol on
suppression of Fusarium wilt of watermelon
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Phytopathology 101:S72

Fusarium wilt, caused by Fusarium oxysporum f. sp. niveum (FON) has resurfaced as an economically important watermelon disease due to the loss of the use of methyl bromide fumigant, and the increase in production of triiploid cultiuvar. Cover crops have shown promise in reducing Fusarium wilt. For example the soil-incorporated cover crop Vicia villosa has suppressed Fusarium wilt of watermelon, but the mechanism of suppression is not known. We measured soil respiration following incorporation of a V. villosa cover crop to determine if it conferred general suppression. We also evaluated cover crops V. villosa, Trifolium incarnatum, Secale cereale, and Brassica juncea, and no cover, alone and in combination with Actinovate biocontrol (Streptomyces lydicus) for induction of suppression. In 2009 Actinovate significantly increased marketable fruit yield in plots inoculated with FON compared to plots without Actinovate, or plots with no inoculation; however in 2010 this effect was not seen. Fusarium wilt incidence was not significantly different among treatments in 2009 or 2010. Measurements in both years indicated that incorporation of T. incarnatum residue significantly increased the rate of soil respiration at the beginning of the field season compared to V. villosa and other cover crops. Because wilt suppression has been reported for V. villosa but not T. incarnatum, this may imply that disease suppression is not general.

The identification and characterization of genes involved in foliar
infection of maize by Cercospora zeae-maydis
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Phytopathology 101:S72

Gray leaf spot, caused by Cercospora zeae-maydis, is one of the world’s most devastating foliar diseases of maize. Despite the impact of this disease, little is known about the interaction between C. zeae-maydis and maize at the molecular level. The recent discovery and characterization of CRP1, a putative ortholog of the White collar-1 family of fungal blue-light photoreceptors, established a linkage between the perception of light and the infection of the pathogen through stomata. To further dissect pathogenesis at the molecular level, laser-capture microdissection is being utilized to isolate fungal tissue during infection, specifically hyphae approaching stomata, nascen appressoria, and mature appressoria. From these samples, the transcriptome of the pathogen is being obtained with next-generation sequencing technologies (RNA-seq) and analyzed to identify genes associated with specific stages of the infection process, such as stomatal tropism and appressorium formation. Candidate genes will be disrupted through targeted mutagenesis to determine their specific roles in pathogenesis. Regulatory genes characterized in this study will further elucidate the genetic mechanisms underlying foliar infection in C. zeae-maydis and related filamentous fungi.

Incursion of Myrtle rust in Australia caused by Uredo rangeli
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Phytopathology 101:S72

On April 25 2010 a new rust of Myrtaceae was detected in a nursery on the central east coast of New South Wales, Australia. The pathogen was subsequently identified as Uredo rangeli belonging to the ‘guava rust complex’. Given the wide host range of pathogens in this complex and the large number of Eucalyptus and other native Myrtaceae in Australia, an extensive campaign was established to slow down the spread of the disease. However, it has been found on many properties and in the bush in NSW and Queensland to the point that it is now technically feasible to maintain this campaign. Governments and industry are now in the process of transitioning to long term disease management to mitigate the impact on the natural environment, including endangered species and industries that rely on Myrtaceae. The knowledge of this disease and its potential impact in Australia is very limited. Taxonomic studies are underway to determine the exact relationship of this pathogen within the guava rust complex. It has now been identified on 55 species of Myrtaceae but further seedling testing suggest that most Myrtaceae might be susceptible to the disease although there are some indications of useful sources of host plant resistance. Other studies focus on modelling environmental impact based on current data. We are seeking science collaborations to broaden our understanding of the ecology and behavior of this disease relative to other members of the guava rust complex.

Biorational control of bacterial wilt of tobacco via induced resistance by
dependent on pathogen
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Phytopathology 101:S72

Plant growth-promoting endophytic bacteria, Pseudomonas spp., significantly reduced the disease incidence and severity of bacterial wilt of tobacco caused by Ralstonia solanacearum. By means of real-time quantitative polymerase chain reaction (qPCR) using specific primers targeted 16S rDNA region of Pseudomonas spp., the bacterial population in tobacco plants was quantified. Pseudomonas spp. colonized the plant root up to 10^6 cfu per gram fresh weight. Transcriptomic studies are underway to determine the exact mechanism of suppression. For example the soil-incorporated cover crop Vicia villosa has suppressed Fusarium wilt of watermelon, but the mechanism of suppression is not known. We measured soil respiration following incorporation of a V. villosa cover crop to determine if it conferred general suppression. We also evaluated cover crops V. villosa, Trifolium incarnatum, Secale cereale, and Brassica juncea, and no cover, alone and in combination with Actinovate biocontrol (Streptomyces lydicus) for induction of suppression. In 2009 Actinovate significantly increased marketable fruit weight. Real-time quantitative reverse transcription-PCR (qRT-PCR) analysis on the expression of defense-regulatory genes Coi1, NPR1 and EREBP and on the down-stream defense genes PRI (PR-1a and PR-1b) and PDF1.2 in the tobacco leaves was carried out to determine the nature of the resistance induced by Pseudomonas spp. The expression of PRI gene, related to the salicylic acid (SA independent pathway, was highly up-regulated in the leaves after dipping the roots in the suspension of Pseudomonas spp. at the concentration of 10^9 cfu per ml for 24 h. However, no significant change in the expression of the PDF1.2 gene, related to the SA independent pathway
and the selected defense-regulatory genes was found. Furthermore, Pseudo-
monas spp. was not able to reduce the wilt incidence in the NaHglytransgenic line (defective in SA accumulation). Our results indicate that resistance in tobacco against *R. solanacearum* induced by *Pseudomonas* spp. is associated with the systemic induction of PR proteins in the SA dependent pathway.

**Improving the detection of new and emerging pests and diseases through the Plantwise Initiative**

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Phytopathology 101:S73

The high percentage of the world’s crops lost to pests and diseases seriously impacts on global food security. CABI is working with a network of international partners, including those from the CGIAR, regional plant protection organisations and governments, to create a new system that can help detect, monitor and control emerging pests more effectively, particularly in the developing world. This Plantwise Initiative contains a central, open access Knowledge Bank to collate information on the detection, identification and effective control of thousands of pests and diseases. Plantwise also delivers appropriate scientific knowledge to farmers in developing countries through plant health clinics held in local markets. Here farmers get face-to-face advice from Plantwise trained ‘plant doctors’ who can also collect local pest information to pass back to the Knowledge Bank. Researchers can then easily access on-the-ground data to improve pest modelling systems while the ‘doctors’ can retrieve relevant fact sheets for the pests encountered. This process helped identify, and provide advice on, the first incidence of Citrus Black Spot caused by *Gaiguardia citricarpa* in Uganda and the planned increase in Plantwise clinics, from 80 to 400 over the next five years, will create much improved reporting of emerging problems. The Knowledge Bank, updated with these records and all new pest distribution data extracted from the scientific literature, will be a core resource on emerging pests.

**The National Plant Diagnostic Network: First detector training and education**


Phytopathology 101:S73

Invasive arthropods, plant diseases, and weeds cost U.S. agriculture billions of dollars annually through direct pest damage and indirectly via eradication and management programs. Each year, integrated pest management (IPM) programs are disrupted by the threat of new, unwanted invaders. In order to raise awareness concerning the threat of invasive species and appropriate sampling as well as communication protocols, the NPNP launched an extensive First Detector training program in 2003. First Detector training occurs in a 2-day face-to-face training (2003–2011), and a wiki platform-based series of pest information pages (2008–2011). First Detectors completing training receive certificates of completion, and the national First Detector newsletter. Nationwide to date, 10,736 First Detectors have been trained in 821 training sessions conducted by First Detector Educators, and 799 have participated in the E-Learning FD training.

**Is the striped mealybug, *Ferrisia virgata*, a vector of huanglongbing bacterium *Candidatus Liberibacter asiaticus***?

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Phytopathology 101:S73

*Candidatus Liberibacter asiaticus* (Las) is the prevalent species of three different Liberibacter that cause huanglongbing (HLB), the globally most devastating disease of citrus. Two pydellid species, *Diaphorina citri* and *Triozia erytreae* are currently known to transmit this endemic disease. Striped mealybugs, *Ferrisia virgata* (Cockerell) (Pseudococcidae; Hemiptera), were recently collected from Las-infected periwinkle plants in a USHRL greenhouse and tested for the presence of Las bacterium. Positive Las results were found in 46 of 73 (63%) mealybugs sampled using HLBasp primers and probe, and the Las populations were estimated to be 3.11 × 10^4 to 2.32 × 10^5 cells per insect. Additional confirmation was made using conventional PCR with six alternative primer sets targeting different Las loci and by 100% sequence similarity of all seven PCR amplicons. Using qPCR L900 primers that target the prophage genes of the Las genome it was found that the range of Ct values were 15.9 to 29.9 for the infected mealybugs. Infected insects reared on healthy plants for 30 days continued to maintain Las infection with an average L900 Ct value of 21.6. Twenty five mealybugs, collected from non-infected plants, tested negative for Las using all detection methods. The striped mealybug has a wide host range with over 68 plant families and 264 plant species reported. To our knowledge, this is the first report detecting a high titler of Cu. L. asiaticus in the *F. virgata* mealybug.

**Assessment of seed treatments to protect against biological winterkill in winter wheat**

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Phytopathology 101:S73

To assess the efficacy of 11 seed treatments against pathogens associated with winterkill, four non-inoculated trials were planted in north-central Montana and four inoculated trials were planted in Bozeman, MT. Plots in non-inoculated trials were six-rows wide, 4.5-m long, and treatments were replicated six times. Inoculated plots were four-rows wide, 2.5-m long, and treatments were replicated four times. All trials were planted with the cv. Genour, a solid-stem, hard red winter wheat. Trials at Bozeman were inoculated in furrow at planting with oat inoculum infected either with *Fusarium culmorum* (Fusarium crown rot), *Bipolaris sorokiniana* (common root rot of winter wheat), *Pythium ultimum*, *Fusarium oxysporum* *spp.* (speckled snow mold), or *Microdochium nivale* (pink snow mold). For all trials, plant emergence (percent stand), green-up vigor, plant height and yield were measured. Additionally, plant crown samples were collected in the springtime from non-inoculated plots for fungal isolations. Pink snow mold caused the greatest winterkill in inoculated trials with non-treated inoculated controls having a 98% stand reduction. Only one treatment, BASF’s Charter/AgriGard/Access/Stamina had a significantly higher percent stand and yield compared to the non-treated inoculated control. This product also yielded the most across inoculated trials. Fungi isolated from non-inoculated trials were dominated by two *Fusarium* species, *F. acuminatum* and *F. tricinctum*, and a common soil saprophyte *Mortierella elongata*.

**Fungal community analysis in wheat residues infested with *Fusarium pseudogaeurinum* through internal transcribed spacer region (ITS) sequencing**

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Phytopathology 101:S73

*Fusarium pseudogaeurinum* is dependent on infested wheat crown residues for survival between cropping cycles. It is hypothesized that *F. pseudogaeurinum*’s dependence on these residues is crucial to its survival. To test whether *F. pseudogaeurinum* actively manipulates fungal communities within wheat crown residues, twelve plots of each two spring wheat cultivars, Chouteau and Outlook, were inoculated with *F. pseudogaeurinum* oat inoculums and six plots were left non-inoculated. Crowns were collected eight months post-inoculation and their DNA extracted. *Fusarium pseudogaeurinum* populations were quantified for these samples using qPCR. Additionally, fungal internal transcribed spacer region (ITS) sequences were amplified from each DNA sample using the primers ITS-1F and ITS-4, bulked together based on treatment and cultivar, and then cloned into E. coli TOP10 cells. A hundred clones per treatment were sequenced and representative sequences for each operational taxonomic unit (OTU) were identified through BLAST-n analysis. Fungal communities from inoculated plots were significantly different from those in non-inoculated plots (Unifrac analysis, p<0.001), and more diverse (Shannon diversity index = 3.15 inoculated versus 2.21 uninoculated). Further exploration of this hypothesis is ongoing using samples from additional locations analyzed with 454 sequencing.

**The biofumigation potential of *Brassica juncea* against black shank of tobacco**

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Phytopathology 101:S73

Kentucky is the nation’s leading producer of burley tobacco and the crop’s most economically important disease is black shank, caused by *Phytophthora nicotianae* (Pn). Current management is effective, however, problems with expense and pathogen persistence are issues. A potential alternative control method, biofumigation, involves incorporating fresh brassica biomass into the
soil where brassica-produced glucosinolates are converted into volatile antimicrobial compounds. To evaluate the potential of biofumigation as a management tool for black shank in the field, a mustard (Brassica juncea) cover crop was incorporated into soil versus bare ground in 2008 and wheat cover in 2009–10. Results demonstrated that populations of Pn in soil were lower for mustard than bare ground in 2008, whereas no significant differences were found between mustard and wheat in 2009 and 2010. Significant differences in disease progression were not seen for any treatment in 2008–10. Greenhouse experiments compared mustard and wheat biomass incorporation at varying levels (0, 10, 50, 100, 200, and 490 g per 0.03 m² of soil), while rates comparable to our field study (100 g) did not reduce survival of Pn. mustard significantly suppressed three microbial agents in microbial fungicides used for the control of Phytophthora blight in the greenhouse test, the control values were in the range of 71.8% to 87.9% when microbial fungicides (B. subtilis DBB1301, B. subtilis QST-713) and chemical fungicide (trifloxystrobin) for the control of Phytophthora blight and powdery mildew were examined in vitro and under greenhouse condition. Five chemical fungicides significantly suppressed three microbial agents in microbial fungicides used for the control of powdery mildew. Also, two microbial agents for the control of Phytophthora blight were affected by 6 chemical fungicides in vitro. In the greenhouse test, the control values were in the range of 71.8% to 87.9% when microbial fungicides when microbial fungicides were used. Also, two microbial agents for the control of Phytophthora blight were affected by 6 chemical fungicides in vitro.

Phytophthora species identified from streams in Virginia


Phytophthora species identified in a nursery irrigation runoff water containment basin of eastern Virginia

Capture and use of agricultural runoff water in containment basins for irrigation is of strategic importance to the ornamental horticulture industry in the light of growing global water scarcity. However, this practice may recycle destructive plant pathogens. The primary objective of this study was to determine the diversity of Phytophthora species present in a containment basin at an eastern Virginia nursery. Whole leaves of Rhododendron catawbiense cv. ‘Boursault’ were used as baits and placed mostly in surface water at different locations for 7 days. Baits were retrieved, rinsed and transported in a cooler to the lab. Recovered leaves were surface-sterilized in 0.525% hypochlorite for 30 seconds, rinsed in deionized water twice then plated in 10-cm Petri dishes with PARP-V8 and PARPH-V8 agar. Emerging colonies were identified to species level using colony PCR-SSCP, morphology and DNA sequencing. The baiting was performed from 2005 to 2008 at monthly intervals for the first year and quarterly thereafter. A total of 21 Phytophthora species were identified. These include P. aquimoribida, P. cactorum, P. citrophthora, P. cryptogea, P. gonapodyides, P. hydropathica, P. insolita, P. irrigata, P. inundata, P. megasperma, P. nicotianae, P. pini, P. polonica, P. pseudodyngiare, P. sansomeana, P. syringae, P. tropicalis and several new taxa. The implications of finding such diverse Phytophthora species in a single basin is discussed.

Phytophthora species identified from streams in Virginia


Phytophthora species identified in a nursery irrigation runoff water containment basin of eastern Virginia


Phytophthora species identified in a nursery irrigation runoff water containment basin of eastern Virginia

South American Leaf Blight of rubber tree: Dynamics of pathogen inoculum, progress and damages, in three topographical strata
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Phytopathology 101:S75

The South American Leaf Blight of rubber tree (SBAL), caused by Microcystis ulmi, is the major limiting factor for rubber production in Brazil. Between June 2005 and December 2008 we studied the: i. dynamics of ascospores and conidia, ii. host phenology and disease progress and iii. effects of height, disease severity, and leaf density on growth and yield of rubber trees. Experiments were set in commercial rubber plantations, in three topographical strata: top, hillside, and lowland. In each stratum, boxes were placed to collect leaves. Disease severity; stoma occurrence and incidence on fallen leaves; leaf wetness; disease prevalence of leaves in stages B, C, and D were evaluated weekly. Starting July 2008, a Burkhard spore trap was installed in each stratum and weather variables were registered. Ascospores and conidia were trapped throughout the experimental period. Higher number of hours with leaf wetness and minimum relative humidity were registered in the lowland, and based on path analysis, these variables influenced SALB severity. Height affected directly and positively, and severity directly and negatively, both rubber production and growth in all strata. Under favorable weather conditions both ascospores and conidia were produced throughout the year suggesting that a review of the life cycle of the pathogen should be conducted. Planting of clones with horizontal resistance is anticipated to be the most effective control measure.

Development of a simple and practical detection method of seed-borne bacterial pathogens from potato tubers
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Phytopathology 101:S75

Of the seed-borne bacterial diseases of potato, black leg disease caused by Pectobacterium atrosepticum (Pa), P. carotovorum ssp. carotovorum (Pcc), and Dickeya spp. (Ds), ring rot caused by Clavibacter michiganensis ssp. sepedonicus (Cms), and brown rot (bacterial wilt) caused by Ralstonia solanacearum phylotypes I and IV (Rs) have been important problems for seed potato production in Japan. In this study, we tried to develop a simple and practical detection method of these bacterial pathogens from seed potato tubers at the same time. To enhance the detection sensitivity of the pathogens from potato tubers, incubation of bacterial cells in King'B liquid medium with shaking at 25°C more than two days (for Pa, Pcc, Ds, and Rs) or six days (for Cms) was most effective. Next, we prepared polyclonal antibodies (IgG) against each bacterial pathogen and used them to detect each bacterial species from artificially infected potato tubers. In combination with enrichment (pre-incubation in King’B liquid medium with shaking 25°C for 24 h) and multiplex PCR method (Bio-PCR), we could detect each black leg pathogens (Pa, Pcc and Ds) and Rs with high level of sensitivity (>10^2 cfu/tuber). Similarly, we tried to detect these pathogens from the potato tubers in combination with enrichment and PCR method (Bio-PCR). Consequently, each species-specific DNA band for Pa, Pcc, Ds and Rs could be amplified from the potato samples infected at lower concentration (10^4–10^6 cfu/tuber). A Cms-specific band also could be amplified, but its detection limit was >10^5 cfu/tuber.

Microplate assay for copper resistance in Xanthomonas spp.
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Phytopathology 101:S75

Copper-containing compounds are the primary means of control for bacterial diseases of tomato; however, field populations of copper-tolerant bacterial strains may increase substantially after repeated sprays of copper. The objectives of this study were to develop a high throughput screening method for monitoring copper resistance in field populations of bacteria, and to isolate bacterial strains that are resistant to high levels of copper (1.2 mM) for further experimentation. Isolates of Xanthomonas spp. were collected from symptomatic plant tissue; a disease survey of tomato fields in Tennessee and Texas was conducted. The assay utilized conversion of resazurin to resorufin, a reaction that occurs in the presence of resiping cells, as an indication of cell viability. Bacteria and a liquid growth medium containing resazurin were added to each well of a 96-well microtiter plate. The effect of copper sulfate, at final concentrations ranging from 0.2 to 1.2 mM, on bacterial growth was tested. Microtiter plates were incubated at room temperature for 24 h. Samples were removed from selected wells and cultured on solid media with and without copper. Absorbances (595 nm and 490 nm) were measured for wells not sampled for bacteria. Absorbance values correlated well with bacterial population counts made from the microtiter plate wells. Isolates capable of growth on solid medium with 1.2 mM copper were recovered for future studies.

Distribution and population density of tea root lesion nematode (Pratylenchus loosi) in Iran
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Phytopathology 101:S75

Tea (Camellia sinensis) is an edible and evergreen plant which has a medicinal and caltive characteristic. At present tea root lesion nematode (Pratylenchus loosi) is one of the most important crop loss agents in Iran, which cause loss in quantity and quality of tea. In this study, distribution and population density of this pathogen was carried out. To investigate these aims, during 2010 to 2011 about 170 complex sample (soil & feeder roots) were collected from all of tea plantation regions in Iran. Sampling was done based on international acceptable generic soil sampling and satellite maps (Google Earth) and GPS. Nematodes were extracted from root samples with Cooien & d’Herd techniques (1972) and were counted by using of counting slide. Total result showed that 86.7% of samples were infested by Pratylenchus loosi. Range of population density was 0.6 to 884 nematode in one gram of feeder roots. Among of the infested samples, 94.2% population density were over than 1 nematode/each gr of root and 5.8% less 1 nematode/1g of root. As though the average population in each gram of infested feeder roots was 97.04 nematode.

Evolutionary and epidemiological consequences of using host resistance genes for controlling tomato spotted wilt virus
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Phytopathology 101:S75

The rapid evolution of resistance-breaking in plant viruses threaten the durability of host resistance genes in crops. To understand the impact of resistance genes on the epidemiology and evolution of resistance-breaking, we conducted an experimental evolution study to determine if pathogenicity and virulence increase during subsequent passages in hosts carrying a resistance gene. Sequential passages through hosts without a resistance gene were expected to result in the loss of resistance-breaking. Resistance-breaking isolates of tomato spotted wilt virus (TSWV) were sequentailly transferred through pepper (Capsicum annum) host lines of TSWV-resistant and susceptible varieties. The change in pathogenicity and virulence was measured in resistant and susceptible varieties across multiple passages in a single host type (resistant or susceptible). Pathogenicity and virulence of resistance-breaking isolates increased with the number of passages in hosts carrying the resistance gene. Consistent exposure to the TSWV resistance gene can drive the evolution of resistance-breaking virus isolates in a population. If resistant varieties are absent, resistance-breaking should rapidly decline in the population. IPM strategies should consider the epidemiological consequences of using resistant varieties in areas where resistance-breaking is present.

Characterization of the roles of the putative secreted protein-encoding XAC1496 in the growth and pathogenesis of Xanthomonas citri subsp. citri
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Phytopathology 101:S75

Xanthomonas citri subsp. citri, a causal agent of citrus canker, is one of the most economically important pathogens in citrus production. A hypothetical gene XAC1496 encoding a secreted protein in X. citri was characterized. Mutation of the gene had no effect on swimming and swarming, and effects of copper on the growth in planta. The gene XAC1496 with EZ-Tn5 transposon conducted an experimental evolution study to determine if pathogenicity and virulence increase during subsequent passages in hosts carrying a resistance gene. Sequential passages through hosts without a resistance gene were expected to result in the loss of resistance-breaking. Resistance-breaking isolates of tomato spotted wilt virus (TSWV) were sequentially transferred through pepper (Capsicum annum) host lines of TSWV-resistant and susceptible varieties. The change in pathogenicity and virulence was measured in resistant and susceptible varieties across multiple passages in a single host type (resistant or susceptible). Pathogenicity and virulence of resistance-breaking isolates increased with the number of passages in hosts carrying the resistance gene. Consistent exposure to the TSWV resistance gene can drive the evolution of resistance-breaking virus isolates in a population. If resistant varieties are absent, resistance-breaking should rapidly decline in the population. IPM strategies should consider the epidemiological consequences of using resistant varieties in areas where resistance-breaking is present.

Characterization of the roles of the putative secreted protein-encoding XAC1496 in the growth and pathogenesis of Xanthomonas citri subsp. citri
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Phytopathology 101:S75
Phytopathology 101:S76

Monilinia species in China—Surprising facts
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Phytopathology 101:S76

Between 2008 to 2010, brown rot samples from nectarines or peaches were collected from peach growing areas in Beijing, Shandong, Zhejiang, Fujian, Shanxi, Gansu, Hubei and Yunnan provinces in China. Cultural, morphological and molecular characteristics of Chinese Monilinia isolates and European or North American isolates were recorded and analyzed. One Chinese species was morphologically most similar to M. fructigena from Europe, however, most of the isolates started to develop stroma after 10 days of incubation on PDA at 22°C while no stroma was observed in any of the M. fructigena from Europe. Another Chinese species most closely resembling M. laxa often developed more than two germ tubes per germinating conidium while Western M. laxa isolates only developed one germ tube per conidium. In pathogenicity tests, lesion growth rates, sporulation, and symptoms on peach fruit were different between Chinese and Western species. Blast analysis of the ITS sequences exhibited eleven, eight sporulation, and symptoms on peach fruit were different between Chinese and European or North American isolates. The phylogenetic analysis based on sequences of two taxonomically informative genes G3PDH and TUB2 indicates that the Western species. Blast analysis of the ITS sequences exhibited eleven, eight sporulation, and symptoms on peach fruit were different between Chinese and European or North American isolates. The phylogenetic analysis based on sequences of two taxonomically informative genes G3PDH and TUB2 indicates that the Chinese species were individually grouped into different lineages from M. fructigena and M. laxa. Our data suggest that the Chinese species may be new Monilinia species, and a new molecular tool was developed to distinguish Chinese from European and American Monilinia species.

Duplex qPCR assay to detect and quantify pathogenic Guignardia citricarpa and non-pathogenic G. mangiferae in plant samples
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Phytopathology 101:S76

The fungal citrus pathogen Guignardia citricarpa causes the potentially devastating disease, black spot, that primarily affects fruit. The disease is found in many humid sub-tropical citrus production regions. It was discovered in Florida in 2010 and quarantine measures are in place to prevent further spread. A nonpathogenic species, G. mangiferae, is an endophytic fungus with a wide host range, including citrus, and is found worldwide. These two Guignardia species are frequently isolated from citrus and have been confused with each other for many years, especially the indistinguishable ascospores. A duplex TaqMan™ qPCR assay was developed to simultaneously detect and quantify these two Guignardia spp. Specific primers and probes labeled with Hex (G. citricarpa) and FAM (G. mangiferae) were designed from polymorphic regions of the internal transcribed spacer (ITS) and actin gene, respectively. The qPCR assay was specific and did not amplify DNA from 6 common pathogens. The detection limits for the two Guignardia spp. was 2 copies of the target sequence inserted into the pDrive cloning vector and 100 fg of genomic DNA, respectively. The two primer pairs had high efficiencies and were not affected by the presence of citrus DNA extracts. Cycle threshold (Ct) values were linearly correlated with the concentration of the target DNA. The duplex qPCR assay described in this study will be a useful tool for quarantine measures and epidemiological research.

Genetic diversity of antagonistic Bacillus subtilis against citrus canker bacteria
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Phytopathology 101:S76

Bacterial canker of citrus caused by Xanthomonas axonopodis pv. citri resulted in great yield loss of various citrus cultivars worldwide. No known biocontrol agent has been recommended for this disease. To explore the potential of bacilli native in Taiwan for control of this disease, Bacillus species with broad spectrum of antagonistic activity against various phytopathogens were isolated from culture substrate and soils. Seven strains including TK1-1, OF-16, SP4-17, HSP1, WG6-14, TLB7-7 and WP8-12 which showed superior antagonistic activity were chosen for biofungicide development. The genetic identity based on 16S rDNA sequences indicated that seven native strains were close relatives of the B. subtilis group which appeared to be discrete from the B. cereus group. The DNA polymorphisms of strains WG6-14, SP4-17, TK1-1, and WP8-12 as revealed by repetitive sequence based PCR with BOXA1R primer were similar while different from those of respective type strains. However, molecular typing of the strains with either DNA- intergenic spacer region or 16S-23S intergenic transcribed spacer region was unable to differentiate the strains at species level. For strain WG6-14, the effectiveness of controlling citrus canker infection has been demonstrated and an endospore formulation has been officially recommended for controlling bakanae disease of rice in Taiwan. Information obtained from molecular typing would provide DNA fingerprints valuable for patenting or commercializing these bacilli strains.

Search for the volatiles of Bacillus cereus C1L involved in the induction of systemic disease resistance and plant growth promotion
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Phytopathology 101:S76

Bacillus cereus C1L was isolated from the rhizosphere of Formosa lily (Lilium formosanum Wall.) in Taiwan. Application of B. cereus C1L effectively decreased disease severity of Botrytis leaf blight in different kinds of lilies, southern corn leaf blight, and the gray mold disease of tobacco; in addition, B. cereus C1L significantly promoted the growth of maize and tobacco. The growth promotion effects were also observed in L. formosanum and Arabidopsis. To find the active compounds for plant growth promotion and the induction of systemic disease resistance, effects of the volatiles of B. cereus C1L on the growth of tobacco and Arabidopsis were analyzed and positive effects were observed. Dimethyl disulfide was identified to be one of the volatile compounds produced by B. cereus C1L, and effective to reduce severity of diseases caused by Botrytis cinerea in tobacco and Cochliobolus heterostrophae in maize. However, significant inhibition of dimethyl disulfide on the mycelial growth of B. cinerea and C. heterostrophae was not observed. Thus, we presumed that dimethyl disulfide could be an active compound of B. cereus C1L to induce systemic disease resistance of plants, such as tobacco and maize. In addition, dimethyl disulfide was also active to promote plant growth as demonstrated in tobacco.

The rice blast fungus, Magnaporthe oryzae, copes with plant-generated reactive oxygen species through the Virulence factor MolyH1
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Phytopathology 101:S76

Reactive oxygen species (ROS) are antimicrobial compounds and appear to be active in the critical zone where pathogens and plants come in contact. During plant-pathogen interactions, the plant may mount several types of defense responses to either block the pathogen completely or ameliorate the amount of disease. A successful pathogen will likely have its own ROS detoxification mechanisms to cope with this inhospitable situation. We focus on one potential fungal virulence factor MolyH1 from the rice blast fungus Magnaporthe oryzae, and its role in ameliorating effects of plant-produced ROS. MolyH1 contains a glutathione peroxidase domain and its yeast homologue was reported to be a thoredoxin-dependent phospholipid peroxidase that detoxifies phospholipid peroxides by forming an inter-molecular disulfide bond with YAP1. We observed that the fungal mutants lacking this gene had a decreased ability to tolerate ROS generated by a susceptible plant, including ROS found associated with cell wall appositions (CWAs). Moreover, deletion of this gene caused a virulence defect in M. oryzae, which we believe is associated with the mutant’s inability to detoxify ROS. Together, our data suggest that HYR1 is a virulence factor in the rice blast pathogen, and its role in virulence is directly related to sensing and managing plant-generated ROS during early infection events.

The amplification culture of endospore formulation of Bacillus subtilis biofungicide and its use in disease management
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Phytopathology 101:S76

The antagonistic Bacillus subtilis has long been used as probiotic biofungicide for the control of various fungal and bacterial diseases. The field application of this biofungicide for disease management in Taiwan, however, was far from extensive mainly due to the cost effectiveness of the commercially available products. In order to reduce the cost of biofungicide production and make it available to the consumers, it was reported to be a thoredoxin-dependent phospholipid peroxidase amplification culture system was developed. With the use of traditional fermentor produced B. subtilis biofungicide preparation as a seed inoculum, and the use of common agricultural waste as major constituent of growth medium, the amplification culture was performed with a self-constructed open tank system where in a timer controlled stirrer and a temperature controlling device was equipped. In a non-sterilized condition, the developed system amplified the seed inoculum by thirty times. The yield of endospores reached approximately 10^10 cfu/mL 10 days after inoculation. For disease control
application, the efficacy of the amplified preparation on the control of citrus canker (Xanthomonas axonopodis pv. citri) was shown comparable to that by fermentor produced biofungicide. The success of the amplified preparation for disease control warrants the extensive and repetitive application of the attempted biofungicide in the routinely practiced cultural management.

Development of genome-based diagnostic markers to detect and differentiate strains of Xylella fastidiosa

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Xylella fastidiosa causes many economically important diseases including pierce’s disease (PD) of grapevine, citrus variegated chlorosis (CVC) and almond leaf scorch (ALS). It also causes bacterial leaf scorch (BLS) of forest and/or ornamental trees and shrubs such as oak, elm, mulberry, sycamore and oleander. At present, complete or draft genomes of seven strains of X. fastidiosa are available in public databases, including the CVC strain 9a5c, PD strains Temecula 1 and GB514, ALS strains Dixon, M12 and M23, and oleander BLS strain Ann 1. By using comparative genomics, these genome sequences were exploited to reveal unique and distinguishing features for identification of highly specific diagnostic markers for target strains of X. fastidiosa. Unique sequences were identified by comparing blastN alignments of defined segments of each respective genome against the other genomes. PCR primers were designed and tested for amplification of DNA sequences from targeted and related strains of X. fastidiosa, with the goal of developing highly specific, easy to use, genome-based approaches to detect and differentiate strains of X. fastidiosa for use in epidemiological studies and in diagnosis and management of diseases caused by X. fastidiosa.

"Peak", a nutritional formulation to suppress bacterial plant diseases

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There are limited options available to control bacterial plant pathogens. Bacteria require certain nutrients for pathogenesis, and plants are dependent on micronutrients as the regulators of defense reactions. “Peak” is a special formulation of beneficial and essential nutrients that is inhibitory to many copper and antibiotic plant pathogenic bacteria. Field trials of “Peak” have demonstrated its efficacy as a plant nutrient and to suppress plant diseases caused by species of Erwinia (fire blight of apple and pear), Clavibacter (Goss’ wilt of corn), Pseudomonas (angular leaf spot of strawberry), Xanthomonas (citrus canker), and Candidatus Liberibacter (HLB, greening of citrus). “Peak” is absorbed through roots, stems, and foliage to provide systemic control of bacterial pathogens within plant tissues. Application timing, rate, and water volume are important considerations to maximize “Peak” efficacy for bacterial disease suppression. A single application of 100 gm a.i/ha provides effective control of most extracellular bacterial pathogens infecting parenchyma tissues. Intracellular bacteria, such as Ca. Liberibacter spp., require several applications for suppression of this phloem-limited bacterium. A single application at mid-bloom has provided control of fire blight of apple comparable to or better than pre- and mid-bloom applications of Mycoscid (oxytetracycline), Agri-strep (streptomycin sulfate) or Bloomtime FD TME325 (Pantoea agglomerans E325).

Genetic diversity of environmental and clinical strains of the Enterobacter cloacae complex determined by multilocus phylogenetic and genome analyses


Enterobacter cloacae is the causal agent of plant disease on a variety of hosts, as well as the cause of opportunistic infections in immunocompromised humans. However, the genetic relationships between environmental and clinical E. cloacae strains have not been determined. We used multi-locus sequence analysis (MLSA) of nine housekeeping genes, to determine phylogenetic relationships among 30 E. cloacae strains from onion, other plants, and humans. Preliminary analyses with a subset of strains and three genes with a similar phylogeny were used to select five E. cloacae isolates from plants form a clade which is distinct from clinical isolates. In order to further study the relatedness of these strains, we have sequenced the genome of E. cloacae EcWSU1, which causes Enterobacter bulb decay on storage onions. EcWSU1 has a circular chromosome of 4.8 Mb and a megaplasmid of 0.6 Mb for a total genome size of 5.4 Mb, which is similar in size to previously sequenced E. cloacae strains. The genomic sequence of EcWSU1 is on average about 85% similar to the sequence of E. cloacae ATCC 13047, a clinical isolate from spinal fluid, and about 98% similar to E. cloacae P101, an endophyte of switchgrass. This information and the preliminary multilocus phylogenetic data has led to a genome comparison study of EcWSU1 and P101 with other E. cloacae strains to determine exactly how closely related the environmental E. cloacae are to the clinical strains.

Fluorescence spectra and lifetime of relevant weed species as impacted by selected herbicides

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In the present study we evaluated the impact of four herbicides of different modes of action (glyphosate, bromoxynil, mesotrione, and amitrole) on the physiological response of four weed species (STEME, SETVIR, CHEAL, and VIOAR), as measured non-destructively by means of pigment fluorescence. As main outcome, we show for the first time the suitability of the laser induced fluorescence technique to evaluate in vivo herbicide-induced physiological changes as revealed by modifications of both fluorescence spectra and lifetime. Alterations of the fluorescence signature depend on the interaction agrochemical-plant species, as well as the time after herbicide application. Measurements in the red and far-red spectral region indicate disturbance in the functionality of the photosynthetic apparatus and chlorophyll concentration, e.g. after application of bromoxynil or mesotrione. Measurements in the blue and green spectral regions reveal changes of both amount and composition of specific fluorophores, i.e. after application of glyphosate and amitrole. The fluorescence lifetime, expressed as LTmean or differentiated in lifetime 1 (short-duration, < 1 ns) and lifetime 2 (long-duration, 5-6 ns) fractions, provided additional information to the spectrally resolved data. In summary, growth parameters were compared in resistant and non-resistant for explorative studies on the action mode of a.i.s in screening programs, dose-response studies, as well as impairment and recovery of weeds and main crops.

Multiplex PCR assay for the simultaneous detection of E. coli O157:H7 and Salmonella spp. from fresh produce

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Pathogenic strains of Escherichia coli and Salmonella cause foodborne diseases if consumed in contaminated produce. In the present study we describe a one step multiplex PCR method for specific detection of E. coli O157:H7 and Salmonella spp. from fresh produce. Three primers targeting different pathogen related genes were prepared and applied for the simultaneous detection of target pathogens. The primers were able to specifically detect the target pathogens from complex culture in the presence of other bacteria as well from the food matrix. The sensitivity of the primers was up to 50 cells per reaction of bacterial culture and 5 pg of genomic DNA. This multiplex PCR offers an efficient microbiological tool for detection of E. coli O157:H7 and Salmonella in fresh produce.

Impact of clubroot resistance on root hair infection, disease severity, and growth of canola in soil inoculated with Plasmodiophora brassicae


Clubroot, caused by Plasmodiophora brassicae Woronin, has become a serious threat to canola (Brassica napus L.) production in western Canada. Several clubroot-resistant canola cultivars have recently been released onto the Canadian market, but resistance needs to be managed carefully because some sources of resistance have broken down quickly in other regions. To improve our understanding of resistance management, root hair infection, clubroot severity, and plant growth parameters were compared in resistant and susceptible canola hybrids grown in inoculated versus noninoculated soil. Primary plasmodia were visible at 4 days after seeding in both cultivars, but root hair colonization, secondary infection, and clubroot severity were always highest in the susceptible cultivar. Plants were shorter when grown in inoculated soils, but the resistant cultivar was taller than the susceptible cultivar on the non-inoculated soil. The effect of repeated cultivation of resistant and susceptible cultivars on subsequent infection levels was studied by growing the cultivars in clubroot-infested soil for six weeks, macerating the galls and putting them back into the soil, and planting the same cultivars in the same soil for two more cycles. At the end of the third cycle, a susceptible cultivar was sown in both soils. Seedling emergence and plant height were lower and clubroot severity was higher, in soil in which the susceptible canola had been grown.
A new model for races of *Xanthomonas campestris pv. campestris*
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The race structure of 165 strains of *Xanthomonas campestris pv. campestris* (Xcc), including 67 strains from known collections and 98 strains from weedy crucifers from coastal California (Ignatov et al, 2008) was determined using a set of seven crucifer differential cultivars, including Just Right F1, Tokyo Cross F1, Seven Top Green, Wiroa F1, Miracle F1 PI199947, and Florida Broad Leaf. Five cultivars of white cabbage-Bejo 2660, Invento F1, Bejo 2659, Jubilee F1, Bronco (Bejo Seeds, NLND), and two breeding lines, PEB1-764 B21P3 (Russian, St. Agirc, University, Moscow), with known resistance to black rot were included. Twenty one Xcc strains were either avirulent or failed to cause systemic symptoms of black rot; 101 belonged to races 1-6, 8 and 9. Race 6 was predominant in the population of Xcc isolated in 2008 from the weedy crucifers. Evidence of new races was found in the interaction of 47 strains with the seven host differentials. Besides previously designated genotypes R1, R3, and R5, three new variants of R4 locus, R4A, R4B, and R4C were identified in turnip cultivars Just Right, Tokyo Cross, and Seven Top Green, respectively. Six matching pairs of avirulence genes in the different strains and resistance genes in the differential cultivars supported a gene-for-gene relationship between the pathogen and host. Evaluation of strain-specific reactions of the seven additional cabbage cultivars support the presence of five new putative avirulence genes in Xcc.

Relative susceptibility of six soybean genotypes against single and multiple virus infections in Nigeria
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Soybean genotypes, TGx1448-2E, TGx1440-1E, TGx1740-2F, TGx1485-1D, TGx1835-10E and TGx1897-10F were evaluated against Cowpea mild mottle virus (CPMMV), Cucumber mosaic virus (CMV), Blackeye cowpea mosaic virus (BCMV), Bean pod mottle virus (BPMV), which are endemic and most frequently occurring viruses in soybean in Nigeria. Tests plants of each genotype were mechanically inoculated at seedling stage with each virus, and also in combinations of two or more viruses. Plants were monitored for symptoms and tested for viruses using ELISA. None of the accessions was found to be immune to the virus treatments, however, genotype response to virus treatments among accessions differed substantially (P < 0.01). High positive correlation was observed between symptom severity and incidence (0.77), plant height (0.77), dry and fresh weight (0.6), number of pods and pod weight (0.97), number of nodes and number of seeds (0.93). Conversely, disease severity was negatively correlated with plant height (-0.52), number of pods (-0.63), pod weight (-0.68), number of seeds (-0.68), seed weight (-0.73). Plants infected with BPMV showed most severe symptoms; whereas CMV had significantly lower influence on performance of genotypes. In mixed infections, CMV-BCMV combination had the highest influence both on plant height and yield. Multiple virus infections of soybeans can result in complete loss of yield. These results underscore the need for the development of multiple virus resistance soybeans appropriate for Nigeria.

Laurel wilt of avocado: Relationships among disease severity, water conduction, and the spatial distribution of *Raffaelea lauricola*
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Laurel wilt of avocado, *Persea americana*, is caused by *Raffaelea lauricola*. Rapid wilting and vascular staining it causes suggest that xylem dysfunction and impaired water transport play roles in the development of this disease. Disease severity, water conduction and the spatial distribution of *R. lauricola* was examined in artificially inoculated plants of 'Simmonds' avocado. Stems were inoculated at 7, 14, and 21 days after inoculation and stained with fuchsin to stain water-conducting tissue; the percentage of functional xylem was inversely correlated with disease severity. Water transport in these stems was also inversely correlated with disease severity. Average water flow rates were 0.6 ml min⁻¹ in severely affected plants vs 200 ml min⁻¹ in mock-inoculated control stems. With a fluorescein-conjugated wheat germ agglutinin stain, *R. lauricola* was observed primarily in discolored, nonconductive areas, usually in close association with tracheids and the walls of xylem vessels. Abundance of the fungus was greatest near the inoculation point and generally decreased as the distance from this point increased. The pathogen was observed in xylem vessels by 14 da. However, complete blockage of the lumen did not occur during the course of the study, nor was there evidence for extensive colonization of the xylem by the pathogen. Although blockage by the fungus may play a role in decreased water conduction in Laurel wilt-affected avocado, additional factors are probably involved.

Histological and ultrastructural changes in avocado (Persea americana) induced by *Raffaelea lauricola*
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*Raffaelea lauricola* causes laurel wilt, which affects avocado (*Persea americana*) and other members of the Lauraceae. Symptoms include the wilting of stems and leaves and vascular staining of the wood. Host-pathogen interactions were examined with light and scanning electron microscopy. The ‘Simmonds’ and ‘Choquette’ avocado cultivars were inoculated and examined at 3, 14, 21, and 42 days after inoculation (dai). Samples were taken from 5, 10, 20 cm acropetal, and 5 and 10 cm basipetal to the inoculation site. At 3 dai, no external or internal symptoms were observed. After 14 dai, external symptoms were present and dark discoloration developed in the xylem tissue of inoculated ‘Simmonds’. Pectic and phenolic compounds accumulated in tracheids and vessels of the stained areas and resulted in the complete blockage of vessels. Tylose formation in the vessels was observed by 14 days, and tylose numbers increased with increasing levels of disease. Mycelia and conidia were observed within lumina of vessels and fibers. In contrast, minor tylose formation and presence of the fungus was evident in ‘Choquette’ at all sample times. Mock-inoculated ‘Simmonds’ and ‘Choquette’ were asymptomatic internally and externally, and tylose free. Understanding these responses will ultimately enhance disease management and host resistance efforts on this crop.

Species limits in *Verticillium*, a group of vascular wilt-pathogens of global importance
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*Verticillium* is a small genus of plant pathogens closely related to *Glomerella* (anamorph Colletotrichum, the causal agents of anthracnose diseases). Like many strains of *Fusarium oxysporum*, *Verticillium* causes vascular wilt that result in severe crop losses. In California, *Verticillium dahliae* affects many different hosts including lettuce, tomato and strawberry. Of lesser importance are *V. albo-atrum*, *V. tricorpus*, *V. longisporum* as well as *V. nubilum* which is only infrequently isolated from diseased plant materials. *Verticillium* spp. form thick-walled, highly melanized resting structures that can survive in the soil for many years. Species identification is largely based on the kind of resting structures produced, i.e., microsclerotia in *V. dahliae*, resting mycelium in *V. albo-atrum*, chlamydospores in *V. nubilum*, and all three kinds of resting structures in *V. tricorpus*. In this study, we used multilocus phylogenetic and morphological analyses of type material and a global sample of *Verticillium* with emphasis on California, to investigate species limits in *Verticillium*. Molecular data indicate that resting structure morphology might be a poor indicator of species limits, as *V. albo-atrum*-like morphology is present in at least two different, unrelated phylogenetic groups.

Atypical ‘deep’ lesions on specialty potato tubers in western Washington caused by *Colletotrichum coccodes*
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Black dot occurs commonly on potatoes throughout the Pacific Northwest of the U.S. Symptoms on tubers typically are superficial brown-to-gray lesions with poorly defined margins that discolor the periderm in circular or irregular patterns. In western Washington in 2009 and 2010, thin-skinned cultivars of Ambra, Chieflain or Yukon Gold displayed deep, sunken, hollow lesions from which isolates of *C. coccodes* (Cc) were obtained. Pathogenicity of one isolate was confirmed on Chieflain and White Rose (3 reps/cv; 3 pots/rep) in a greenhouse test where 7- and 3-wk-old plants, respectively, were inoculated 24 hr after emergence. Colonization was observed by Cc on 1/2 PDA plates, 10 wk prior to use. In France in 2003, Cc caused deep lesions on inoculated tubers kept between 5 and 15°C. Thus, four reps of five field-grown tubers of Ambra with similar percentage of deep lesions were photographed and analyzed for disease severity using APS Assess digital imaging software after placing in paper bags and storing inside plastic boxes with lids at 4, 7, 11, 15 and 20°C. Image analyses on 1/5/10, 12/3/10, 1/7/11, 2/14/11 revealed black dot severity changing from average 38.5% to between 56.7% (for 7°C) and 64.5% (for 4°C); only 20°C treatment had significantly (P =
Control of late blight on tomato in western Washington using high tunnels

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Control of late blight (LB) using high tunnels (HT) was studied near Mount Vernon, 2008 to 2010, by comparing HT vs. open field (OF) grown tomatoes, exposed to natural inoculum of Phytophthora infestans. Six-week-old seedlings of five susceptible (Big Beef, Early Girl, Northern Delight, Oregon Spring, Stupice) and one resistant (Legend) cultivar were transplanted into one or two blocks of HT and OF plots on 6/03/08 or 6/02/09 using four reps of six plants per cultivar. In 2010 four blocks with four reps each of Early Girl, Oregon Spring and Stupice seedlings were transplanted 5/27/10 or 6/3/10 into HT or OF. Plants were drip-irrigated, staked and pruned, and rated weekly for disease. Environmental data were recorded every 15 min. Low LB pressure precluded differentiation between HT and OF plots in 2009. However in 2008, the range of area under disease progress curve (AUDPC) values across cultivars was lower in the HT (0 to <1) vs. OF (71 to 246) blocks. Similarly in 2010, average AUDPC values and percent blighted fruit were significantly (P = 0.001) less in the HT (<1 and 1.1%) vs. OF (344 and 13.2%) blocks. LB disease severity values (dsv) calculated by WISDOM software (UW-IPM) were also lower, number of days to 18 ds v longer, and total hr of leaf wetness less for HT compared to OF all three years. In both 2008 and 2010, fruit yield was higher or significantly higher in HT than OF indicating HT as a desirable tomato cropping system for managing LB in the region.

Implication of early-season fungicide application on season long dollar spot control

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Extended control of dollar spot (DS), caused by Sclerotinia homoeocarpa F.T. Bennett, has been reported with fungicides applied weeks before traditional preventative applications. Field studies were conducted on Agrostis stolonifera L. maintained at 1.3 cm in Connecticut (CT) and Pennsylvania (PA) to assess the effect of an early season fungicide application on DS control programs during 2010. Main effects included preventative fungicide timing (mid-April or late-May application of vinclozolin), summer applied fungicides (chlorothalonil, vinclozolin, or bosalid), and application interval of summer fungicides (14-, 21-, or 28-d). Dollar spot severity in the study areas increased in CT and PA during early- and mid-July, respectively, although results varied by location. In CT, DS was less severe in chlorothalonil and bosalid treated turf relative to DS in soil a 3-wk preventative application in mid-April compared to late-May during July and August, and August respectively. Mid-April preventative application reduced DS severity in turf treated every 21 d compared to turf receiving a late-May application. However, no difference was observed in 14 d treated turf in CT and PA. Additional significant preventative timing effects were not observed in PA. These data suggest early season fungicide applications can improve DS control throughout the season; however this effect appears to be inconsistent among locations.

Potential role of grafting as a method to manage Verticillium dahliae race 2 in tomato production systems

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Fresh market tomato production is one of the largest vegetable economic commodities in North Carolina. More than 2,000 acres are harvested each year across the state. Verticillium dahliae race 2, that causes Verticillium wilt (VW), is the main pathogen that limits productivity in western NC. Host resistance is not available to race 2 as it is for race 1 forcing growers to depend on soil fumigants or abandon the land for tomato production. We hypothesized that preferred scions can be grafted to resistant (RS) that confer plant vigor and associated tolerance to VW damage. Maxiort RS grafted to Mt. Fresh scions reduced VW incidence in open field experiments from over 90% in non-treated plots to 20–30%. Incidence in fumigated controls was less than 10%. However, marketable yield of large, extra-large and jumbo fruit harvested from Maxiort grafted plants was similar to yields from plants in fumigated soils. Grafted plant spacing at 50% of standard spacing generated yields similar to non-grafted plants spaced 46 cm between plants. In a complimentary study using several genetically diverse RS, tomato root and stem tissues were collected to assay the extent of pathogen colonization in the rootstock and scion tissue to discern if the rootstock limits colonization or confers true tolerance. Integrating grafting technologies with other IPM tactics could provide a viable tool in place of, or as a complement to, fumigation for managing Verticillium dahliae race 2.

Fvfsr1 in Fusarium virguliforme affects the development of SDS in soybean

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Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS), an important disease of soybean resulting in significant losses in yields every year. Despite the importance of SDS, a clear understanding of fungal genetic factors that affect the development of the disease is still lacking. We have identified Fvfsr1, a F. virguliforme gene that encodes a protein similar to a family of stratiain proteins previously reported to regulate cell differentiation and ascocarp development in several Fusarium spp. Characterization of the fsr1 in other Fusaria revealed its direct role in pathogenesis. Fvfsr1, the fsr1 homolog in F. virguliforme, was disrupted using a split marker approach. The resulting Fvfsr1 transformatant showed a significant decrease in condiation compared to the wild type. A greenhouse pathogenicity assay was conducted to determine the effect of the disruption of Fvfsr1 on the aggressiveness of F. virguliforme on soybean. The disruption of Fvfsr1 resulted in a significant decrease in SDS incidence and severity in the inoculated soybean plants.

Fusarium wilt of strawberry, caused by Fusarium oxysporum f. sp. fragariae, a new disease in California

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Fusarium wilt of strawberry, caused by Fusarium oxysporum f. sp. fragariae (Fos) was discovered in California in 2008. Dieback caused by Fos has been restricted to fields in which pre-plant fumigation with methyl bromide and chloropicrin has been discontinued. Thus it appears that alternative practices such as bed fumigation with chloropirin and 1, 3-dichloropropene have allowed soil populations of the pathogen to increase to damaging levels. Our objectives are to determine the limits of the infestation in commercial fruit production fields, assess the diversity of the pathogen population, develop an assay for quantification of the pathogen in soil, and assess the efficacy of various pre- and post-plant treatments to minimize the impact of the disease. Although initially limited to Ventura County, infested fields have since been located also in Monterey County, which is within the largest strawberry production district in California. Thus far, the population of Fos in California appears to be comprised of two somatic compatibility groups. Quantification of Fos populations using a soil dilution plate assay, in which the pathogen colonies are identified by their distinctive colony morphology. Control measures being evaluated include adjustments of soil pH to 7.0 or above, the application of Trichoderma in an attempt to reduce the rate of infection, and screening strawberry genotypes for resistance to the disease.

Diversity and fungicide resistance of Phytophthora capsici on vegetable crops in Georgia

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Phytophthora blight caused by Phytophthora capsici has become an increasing concern in vegetable production in Georgia. It is imperative to understand the phenotypic and genotypic diversity and fungicide resistance of the pathogen for more efficient management of the disease. Morphological, physiological, and genetic characteristics of P. capsici isolates from different vegetable crops in Georgia were determined in this study. The results indicated that P. capsici populations in Georgia are diverse. Although isolates from different hosts did not differ morphologically, 12 pathotypes were identified based on their aggressiveness on pepper cultivars. The aggressiveness of the isolates appeared not to be associated with host origin or year of isolation. All isolates grew at temperatures from 10 to 36°C but not at 38°C, and no isolate was able to recover after 5 days of incubation at 38°C. Chlamydospores were not produced by the isolates in liquid culture. The isolates were divided into five groups based on randomly amplified polymorphic DNA analysis, and genetic variations were moderately associated with geographical location and host origin of the isolates. Isolates insensitive to mefenoxam and cyazofamid were identified, but all the isolates were sensitive to fluopicolide and mandipropamid. These studies provide
useful information for designing more efficient programs to manage Phytophthora blight on vegetables.

The host-specific virulence activity of *Ralstonia solanacearum* type three effector PopS

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The bacterial wilt pathogen *Ralstonia solanacearum* injects protein effectors into host plants via a type three (T3) secretion system. The *R. solanacearum* pangeneome encodes over 100 T3 effectors whose virulence functions are largely unknown. PopS, a T3 effector of the AvrE/HopR family, is present in all eight sequenced bacteria in the *R. solanacearum* species complex, including strains with wide and narrow host ranges. These PopS orthologs are 80–100% identical in amino acid sequence and their phylogenetic grouping mirrors that of their strain, suggesting that PopS is a conserved ancestral T3 effector. A comparative transcriptome analysis revealed that two genetically and ecologically distinct *R. solanacearum* strains, GMI1000 (Asian phylootype I) and UW551 (American phylootype II), both highly upregulate popS expression while colonizing tomato stem at the onset of wilt disease. UW551 mutants lacking popS are moderately delayed in virulence on susceptible tomato (*Solanum lycopersicum* cv. Bonny Best) and are more dramatically impaired in virulence on a quantitatively wilt-resistant tomato line (Hawaii 7996). However, the popS mutant had full wild-type virulence on bittersweet nightshade (*S. dulcamara*), an epidemiologically relevant weed host. This suggests that the contribution of PopS to virulence is host-specific. We are characterizing the virulence activity of PopS in diverse *R. solanacearum* strains and determining the effect of PopS on plant defense responses.

Tank-mixing of dodine in early-season apple scab programs and possibilities for renewed use in the eastern U.S.

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In recent years, site-specific fungicide resistant *Venturia inaequalis* (apple scab) populations have become a growing problem in apple orchards throughout the eastern U.S. Few options for managing fungicide resistance are available to growers. The objective of this study was to evaluate the benefit of re-introducing dodine in tank-mixes with captan or mancozeb in orchards where resistance to dodine had been detected. Trials were conducted in several eastern U.S. (e.g. New York, Michigan, Virginia, Pennsylvania and North Carolina) using dodine alone and in mixtures with captan or mancozeb in the early apple season, with at least two applications. It was demonstrated that the use of dodine in tank-mixes did not result in control failures due to reemergence of practical resistance, but provided scab control that was as effective or improved over standard programs of protectant and systemic fungicides managing apple scab. Because of the variability of orchard populations in the tests were composed of sensitive isolates as well as those with reduced sensitivity to dodine, dodine may still prove useful in many orchards in the eastern U.S. This research helps elucidate the uncertainty surrounding the reemergence of practical resistance to dodine and associated crop failure.

Results of long-term trials to control diseases in cereal crops

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The control of weeds, pests and fungal diseases was studied in a long-term trial in two crop rotations (12 years) to contribute to the determination of the necessary minimum of pesticides. Dominant diseases investigated were brown rust (*Puccinia recondita*) and Rhynchosporium leaf spot (*Rhyynchosporium secalis*) in winter rye, net blotch (*Pyrenophora teres*) in winter barley, and Septoria leaf blotch (*Myosphaerella graminicola*) in winter wheat. The severity of these and other diseases was mainly determined by the annual weather-dependent infection pressure and cultivar susceptibility to diseases. Fungicides were used as soon as a certain disease threshold was exceeded. Two intensity levels, 100% (situation-related), and 50% of them, achieved good to very good fungicidal effectiveness, with the one of the lower dosage level often being significantly lower when highly infested. Cultivar resistance proved to be the determining factor of the need for fungicide treatment. In winter rye and winter barley, where resistance to the dominant diseases was rather low, at least one fungicide treatment was necessary in all years studied. In the highly resistant winter wheat cultivar Pegasus, on the other hand, moderate to higher infestation occurred in only three years, and no fungicide treatment was required at all in other three years. Generally, fungicide use was economically beneficial only in years with high infestation levels and weather conditions favourable to yield formation.

Community structure of *Aspergillus flavus* and persistence of the atoxigenic strain *A. flavus* AF36 in applied fields

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Aflatoxins are toxic and carcinogenic metabolites produced by several fungi in *Aspergillus Section Flavi* that frequently contaminate crops. Aflatoxins impact the value of crops. The use of atoxigenic strains of *A. flavus* to displace aflatoxin producers is a proven method to reduce aflatoxin contamination. Previous work indicated applications benefit both treated and subsequent crops. The current study sought to determine factors that influence persistence of AF36 and reestablishment of the highly toxigenic S strain of *A. flavus*. Results indicate significant differences between treated areas for both persistence of AF36 and development of the S strain. The percent of the *A. flavus* community composed of AF36 two years after application was higher in Mohawk Valley (>70%) than in the Yuma Valley (50%), while S strain incidence was higher in the Yuma Valley (>40%) than in the Mohawk Valley (20%). Regression analyses indicate that the Percent AF36 significantly decreased, while the Percent S significantly increased in the Yuma Valley. There was no significant change in either the Percent AF36 or the Percent S in the Mohawk Valley. Crop rotation significantly affects the structure of *A. flavus* communities. Cotton and lettuce production resulted in higher AF36 retention and reduced incidence of the S strain. The results suggest growing season, area, and crop rotation all influence the fungal community structure and long-term influences of the atoxigenic strain treatments.

Cultivation and formulation of an endophytic *Beauveria bassiana* strain

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Endophytes like the entomopathogenic fungus *Beauveria bassiana* play an important role in protecting plants against herbivorous insects. Our aim is to develop a process for fermentation and formulation of *B. bassiana* isolate ATP-04 in order to mass-produce and formulate the fungus in such a fashion that it infects rape plants and protects them from insect pests. *B. bassiana* was raised at 25°C and 150 rpm at pH 5.5 in shake flask cultures to produce submerged conidiospores which are reported to show a higher shelf life than mycelium and blastospores. In mineral media with 5% sugar beet molasses as carbon source, *B. bassiana* strain FL-1195 and closely related bacteria (*Xanthomonas campestris pv. citrulorum*, Syn. *X. axonopodis pv. citrulorum*, Syn. *X. campestris pv. citrulorum*) was increased to 5.1 × 10⁸/mL (8.0 × 10⁸ total spores/mL) in the same time span. Different formulation methods, such as encapsulation, film-coating and spraying were investigated. The radial growth of mycelium out of beads containing 20% Ca-alginat was increased by approximately 30% compared to beads from technical yeast extract or autoclaved baker’s yeast by 8% compared to the beads without yeast. Film-coating of commercial fungicide-treated rape seeds with 2 × 10⁴ spores/seed showed that *B. bassiana* grew on 80% of the seeds. Further experiments will deal with production of submerged conidiospores by fermentation and efficacy tests.

Comparative genomic analysis of *Xanthomonas axonopodis* pv. *citrulorum* strain FL-1195 and closely related bacteria

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*Xanthomonas axonopodis* pv. *citrulorum* (Syn. *X. alfaeae* pv. *citrulononis*, *X. campestris* pv. *citrulorum*) is the causal agent of citrus bacterial spot. XACM was isolated from and is limited to the nurseries of the genus of XACM strain FL-1195 was sequenced using 454-pyrosequencing, Illumina ( Solexa) sequencing and Opengen open mapping. The finished sequence of XACM (4,967, 469 bp) was annotated and curated. Our analysis revealed that xacm lacked plasmids, although they are economically associated with other strains of Xanthomonas. Phylogenetic analysis based on housekeeping genes revealed a close relatnessedness of XACM to *Xanthomonas axonopodis* pv. *citri* strain 306 (XAC) causing citrus canker and *Xanthomonas campestris* pv. *vesicatoria* strain 85-10 (XCV) causing bacterial spot in tomato and pepper. Whole genome comparison revealed a gene order
similar to both XAC and XCV. Several genome rearrangements and insertion/deletion regions indicating genome plasticity were found. An all against all BLASTP of the complete proteomes revealed a total of 410 coding sequences unique to XACM. Comparative genomic analysis showed various changes in genes encoding effectors, cell wall-degrading enzymes, lipopolysaccharides, etc. Further molecular analysis of these features could account for differences in virulence and host specificity of these strains.

Identification, hosts, distribution and molecular phylogeny of desert truffles in Iran
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Phytopathology 101:S81

Desert truffles are the hypogeous ascospor of some ascomycetous ectomycorrhizas, which can be found in semi-arid ranges of Middle East, especially Iran. The present investigation was carried out on the identification, hosts, distribution and phylogenetic relationships of these ectomycorrhizal symbionts in Iran. Among specimens collected from different climatic conditions, Terfezia iclaveryi, Tirmania pinoinyi, T. nivea and Picoa lefleurei were identified in different parts of Iran. T. claveryi was present in most parts of Iran. Truffles usually appear after the rainy season in the months of February to April in Iran. The results of physico-chemical analyses on soil samples from different parts of Fars province in Iran showed that the genus Termania was more prevalent in soil with high CaCO3 and silt percentage than the T. claveryi. The location of survey sites are recorded with GPS as a point or polygon in latitude and longitude. The Canonical Correspondence Analysis (CCA) indicated that soil structure were most important environmental parameter that influenced truffle distribution. Phylogenetic analyses indicated a close genetic relationship between Termania and Terfezia. The field, laboratory and anatomical studies showed that Helianthemum ledifolium, H. salicifolium, H. lipii and Carex stenophyllum have ectomycorrhizal association with the four species in the studied areas.

Validation of real-time PCR assays for bioforensic detection of model plant pathogens
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Phytopathology 101:S81

The U.S. agricultural sector is vulnerable to bioterrorist and criminal threats. To attribute such crimes, law enforcement agencies need forensically valid methodologies to reconstruct these crimes. Validation consisted of testing the PCR assays developed and validated for use in forensic testing. Each assay displayed linear amplification of the target nucleic acid, was capable of detecting as little as 100 fg of target nucleic acid, and was shown to be specific to the target pathogen, virus, or species. Detection of GLRaV-3 specific genomic and subgenomic RNAs and the GFP gene sgRNA in Northern blots further confirmed that the presence of the ectopically expressed silencing suppressors is required for replication of GLRaV-3 minireplicon. Agrobacterium-mediated delivery of the GLRaV-3 minireplicon into Nicotiana benthamiana leaves showed expression of GFP only in leaves co-infiltrated with silencing suppressors. Detection of the GFP gene expression in transgenic leaves using RT-PCR. Detection of GLRaV-3 specific genomic and subgenomic RNAs and the GFP gene sgRNA in Northern blots further confirmed that the presence of the ectopically expressed silencing suppressors is required for replication of GLRaV-3 minireplicon. Agrobacterium-mediated delivery of the GLRaV-3 minireplicon containing different portions of the 5′NTR and monitoring GFP expression in leaves co-infiltrated with silencing suppressors demonstrated that sequences at both ends of the 5′NTR contain elements that are essential for virus replication.

Elimination of small fruit viruses by in vitro therapy
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Phytopathology 101:S81

Germplasm of Rubus and Ribes sp. must enter the U.S. through quarantine, and virus infections in the material delay or prevent its release to breeding/research programs. In vitro pathogen elimination protocols using heat treatment and meristem therapy are being developed for these genera. Axillary buds of virus-infected Rubus sp. were grown aseptically at 4-h alternating periods of 29°C and 38°C (HT) with 14 hr day length for 5 weeks. In vitro explants were tested for Black raspberry necrosis virus (BRNV), Blackberry yellow vein associated virus and Tomato ring spot virus using RT-PCR. RTPCR was used to detect Raspberry bushy dwarf virus and Tobacco streak virus. Elimination of BRNV in these tests (5 weeks HT) prompted experiments where BRNV-infected explants were heat treated from 1 – 5 weeks. After thermotherapy, all induced shoots were grown on culture media, transplanted to soil, and subsequently tested as virus free for 5 months under greenhouse conditions. Testing of additional BRNV-HT plants and other virus infected Rubus sp. continued. Ribes species infected with Gooseberry vein banding associated virus (GVBaV) were grown at 29–34°C due to the high mortality of explants at 38°C. Preliminary data indicates GVBaV may be eradicated or with or without thermotherapy using meristem tips of ≤1 mm. Additional GVBaV-infected explants will be subjected to meristem extraction/heat to validate these results. These protocols can be used to clean up virus-infected germplasm entering the U.S. through quarantine.

Impact of global climate change over the geographic distribution of Ceratocystis fimbriata of eucalyptus in Brazil
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Phytopathology 101:S81

In December 1997 a new disease was observed in clonal eucalyptus reforestation in Brazil, caused by Ceratocystis fimbriata. This disease has caused serious damage to the country, being responsible for more of 40% of the mortality of plants. In this sense, this work had as an objective evaluate the potential impact of global climate change over the C. fimbriata in Brazil. We were prepared maps with the favorability of the climate to the occurrence of C. fimbriata in the current and future period. The future scenarios used (A2 and B2) were centered for the decades of 2020, 2050 and 2080. These scenarios were obtained from six global climate models (GCM)’s provided by

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the Intergovernmental Panel on Climate Change (IPCC). Considering the global warming scenarios provided by the IPCC, it will reduce the potential risk of occurrence of *F. fimbriata* climate in Brazil. Furthermore, the most favorable period of the disease occurrence also will tend to reduce in future decades. These reductions are predicted in both scenarios for the future, but it will occur more sharply assuming the A2 scenario. Additionally, changes in the geographical distribution of the disease will occur from one month to another, with unfavorable areas becoming favorable and vice-versa. However, in spite of these changes, extensive areas will still continue being favorable for the occurrence of *F. fimbriata*, especially in Brazil’s major producing regions.

**Potential impact of climate change over the occurrence of black spot of papaya in State of the Espirito Santo**


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Phytopathology 101:S82

The State of the Espirito Santo is the major producer of papaya of the Brazil. However, this culture is affected by various diseases. Among the diseases caused by fungi, the black spot (*Asperisporium caricae*) is the most important economic. This disease is responsible for causing significant losses in environmental conditions favorable. In this sense, this work evaluated the potential impact of global climate change over the occurrence of black spot of papaya in Espirito Santo. Were prepared maps with the favorability of the climate to the occurrence of disease in the current period and future. The future scenarios used (A2 and B2) were centered for the decades of 2020, 2050 and 2080. These scenarios were obtained from six global climate models provided by the Intergovernmental Panel on Climate Change (IPCC). Assuming future scenarios outlined by the IPCC, will occur reduce in occurrence of climatic favorability of black spot in both future scenarios (A2 and B2). Furthermore, the period of greatest risk of black spot will tend to reduce in future decades (A2 and B2). However, these planned changes will be larger in the A2 scenario compared to the predicted scenario B2. Therefore, changes in the geographical distribution of the disease will occur from one month to another, with favorable areas becoming unfavorable. However, in spite of these changes, extensive areas will still continue being favorable for the occurrence of black spot of papaya in Espirito Santo.

**Identify the pathogen of tomato yellow leaf curl disease of Jiangsu, China**

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Phytopathology 101:S82

In 2007, a new tomato disease occurred in Jiangsu, China, which caused great losses to local tomato production. The result of field investigation and partial sequences analysis shows that the disease associated with a Begomovirus. DNA-A component of the typical isolate (j01) was sequenced and no DNA-B or other component was detected. Sequence analysis showed that the virus shared high identities with tomato yellow leaf curl virus (FJ690655). To investigate the pathogenicity of DNA-A, an infectious clone of j01 was constructed and inoculated tomato. Tomato leaves showed chlorotic and upward curling of the leaflet margin after 15 days, typical symptoms of tomato yellow leaf curl disease, which confirmed the new tomato disease occurs as a typical symptoms of tomato yellow leaf curl disease. This work was supported by Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201003065) and Jiangsu Agricultural Scientific Self-innovation Fund (Grant No. CX[10]415, CX[10]207).

**Identification of pathogens responsible for root rot diseases of wheat and maize in Hebei, China**

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Phytopathology 101:S82

In the North China Plain, semi-annual cropping of winter wheat followed by summer maize is the most common agricultural production system. A shift to retention of maize residues rather than burning has been accompanied by an increase in root rot diseases. In 2008–2010, root rot of maize and wheat were investigated and pathogens were isolated in two successive years. 1464 and 1678 maize plants were assessed for root rot infection at seedling stage with disease incidences 83 and 90% in 2008 and 2009, respectively. *Fusarium verticillioides* and *Bipolaris sorokiniana* were the most common root rot pathogens (44 and 20% of 902 isolates, respectively). Low frequencies of *F. graminearum*, *Pythium sp.* and *Rhizoctonia sp.* (about 3% each), were also found. 2319 and 1233 wheat plants were assessed for root rot diseases at tillering stage in 2009 and 2010, respectively. The diseased root and stem incidences were 72 and 32% in year 2009 while 80 and 73% in year 2010, with 16% (2009) and 12% (2010) of plants having serious root browning, discoloration and stunting. Again, *F. graminearum* (35% of 741 isolates) and *B. sorokiniana* (30%) were most popular pathogen. Moreover, *Rhizoctonia spp.*, *F. incarnatum*, *F. equiseti*, *Pythium spp.* and *F. verticillioides* representing 11, 9, 3, 2 and 1%, respectively. The established host specificity clearly favours the build up of pathogens responsible for root diseases in both wheat and maize. New integrated methods are needed to control root rot diseases in North China Plain.

**Linkage block and recombination suppression at the Pi-ta locus at the centromere region of rice chromosome 12**

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Phytopathology 101:S82

The *Pi-ta* gene, located near the centromeric region of chromosome 12 is an effective resistance gene to *Magnaporthe oryzae* that causes rice blast disease. *Pi-ta* has been incorporated into diverse resistant rice cultivars by classical plant breeding in the southern U.S. and worldwide. Previously, large linkage blocks around *Pi-ta* ranging from 14 to 27 MB were observed in rice cultivars and in backcross progeny derived from an indica x japonica cross. In the present study, the same linkage block was further examined in 1600 random recombinant individuals possessing or lacking *Pi-ta* that were derived from indica x japonica, indica x indica crosses. Simple sequence repeat markers distributed over the centromeric region were used to detect recombination break points and to delimit the physical size of the linkage blocks. Large linkage blocks ranging from 4.1 to 10 MB on chromosome 12 were identified from recombinant individuals of indica x japonica crosses. However, significantly smaller blocks, ranging from less than 400 kb to 1 MB, were identified in indica x indica crosses regardless of the presence of *Pi-ta*. The large linkage blocks previously observed in rice cultivars and backcrossing progeny was predicted to be a result of recombination suppression and selection for blast resistance. These findings suggest that crosses of indica x japonica rice have significant recombination suppression at the centromeric region of chromosome 12.

**Alteration of gene expression profile in maize infected with a double-stranded RNA fijivirus associated with symptom development**

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Phytopathology 101:S82

Maize rough dwarf disease caused by rice black-streaked dwarf virus (RBSDV) is a major viral disease in China. It is suggested that viral infection of maize can cause distinct disease symptoms through inhibiting or activating host gene transcription. We scanned the gene expression profile of RBSDV-infected maize through oligomer-based microarrays to reveal possible expression changes associated with symptom development. Our results demonstrated that various resistance-related maize genes and cell wall- and development-related genes such as those for cellulose synthesis were responsive to virus infection; however these responses were dramatically altered. These results could shed lights to finding new strategies to protect cereal crops against viruses and revealing the molecular mechanisms for the development of specific symptoms in rough dwarf-related diseases.

**Discovering putative *Phytophthora palmivora* disease tolerance genes in papaya (*Carica papaya* L.).**

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Phytopathology 101:S82

*Phytophthora palmivora* is a destructive pathogen of papaya (*Carica papaya* L.). Field study, cultivar SunUp is susceptible while Kiama is tolerant to *P. palmivora*. In this work, a normal distribution of tolerance to *P. palmivora* in F1 (Kamiya crossed SunUp), indicating that the tolerance measured is a quantitative trait. With cutoff of 80% in tolerant F2 and 40% in susceptible F2 plants analyzed with AFLP (amplified fragment length polymorphism) using 130 primers sets, seven polymorphic fragments were linked with disease tolerance. By screening ±2 Mbp upstream/downstream from the fragments to locate them within the papaya genome, five out of seven polymorphic loci were found putative resistance (R) proteins around it. Beside the R proteins, MAP kinase, WRKY transcription factor, hypersensitive-induced reaction (HR) protein, pathogenesis-related (PR) protein, pathogen-inducible ethylene-responsive factor (ERF), multidrug resistance-associated protein (MRP), tobacco rattle virus-induced protein, and avirulence elicitor response (Avr) were found putative resistance (R) proteins around it. Beside the R proteins, MAP kinase, WRKY transcription factor, hypersensitive-induced reaction (HR) protein, pathogenesis-related (PR) protein, pathogen-inducible ethylene-responsive factor (ERF), multidrug resistance-associated protein (MRP), tobacco rattle virus-induced protein, and avirulence elicitor response (Avr)
protein, were also located near the biomarkers that may be used in papaya tolerance to P. palmivora. This study highlights specific functional R genes’ and resistance related genes’ segregation reflected by P. palmivora-resistance related AFLP markers were dominated those candidates pathogen resistance genes from tens/hundreds others members in the gene families.

**Radar observations of the migration of Nilaparvata lugens S. (Delphacidae) in southern China**

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Phytopathology 101:S83

The brown planthopper, Nilaparvata lugens S., is one of the most serious pests of rice in both temperate and tropical regions of east and south Asia. It cannot overwinter in China. The migration regularity and flight behavior of N. lugens were studied by using an 8.8 mm wavelength scanning entomological radar, a searchlight trap and a ground light-trap, field cages, systematic field survey and dissection of female ovarian in 2007and 2009. N. lugens took off at dusk and dawn. The dusk take-off with area density peaking 45 mins later can last 1 h, while the dawn take-off can last 30 mins. After mass take-off, N. lugens climbed up high altitude rapidly. In spring and autumn the flight altitude can reach 2200 and 1800 m respectively. N. lugens formed 3 dense layers which were at heights of 400 to 700 m, 700 to 1000 m and 1100 to 1700 m in spring and 300 to 500 m, 600 to 700 m and 900 to 1100 m in autumn. The thickness of autumn layer was thinner than spring layer. Wind shear was the main reason causing N. lugens forming dense layer, while heavy rain/frost a rising current. Collective orientations of N. lugens with the typical “dumbbell” echo often at the height of 800 to 1200 m on the PPI were observed in the autumn migration. The orientation direction was 159.4 ± 15.14° with an acute angle 53.4 ± 13.74° to the wind direction.

**Mycoviruses that infect plant pathogen Sclerotinia sclerotiorum R genes' and resistance related AFLP markers were dominated those candidates pathogen resistance genes from tens/hundreds others members in the gene families. In this study we analyzed how populations of V. inaequalis changed during Apple scab epidemics in PA in the absence of chemical control. Sampling was done from two cultivars differing in their resistance to Apple scab: ‘Golden Delicious’ (susceptible) and ‘Rome Beauty’ (highly susceptible), at the beginning (May) and near-end (September) of two epidemic years, 2008 and 2009. Eight populations of V. inaequalis (765 isolates) were analyzed using seven microsatellite markers in this study. Overall, in 2008 we observed a significant reduction of genotypic diversity and a dramatic shift in genotype composition from May to September in ‘Rome Beauty’, whereas populations from ‘Golden Delicious’ maintained the same level of diversity throughout the epidemic. However, populations in both cultivars remained stable and did not change significantly in 2009. These results suggest that fitness competition between individuals is more intense on highly susceptible cultivars than on cultivars carrying some resistance genes. We also hypothesize that the pathogen population structure in a given year may be significantly affected by weather and disease pressure during the preceding year.

**Dynamic monitor of physiological race variation for wheat stripe rust in Gansu province in China**

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Phytopathology 101:S83

South Gansu is a variable physiological-race area of wheat stripe rust in China, from where a lot of dominant races are developed and prevail. In Gansu, the race monitoring of wheat stripe rust can provide advanced information for disease forecasting and resistance-breeding. During the period of 2008–2010, there are 977 samples from 30 Gansu counties are monitored and in which 36 races are detected by Chinese differentials. The analysis to virulent gene indicates that the proportion rates of virulent races to Yr-9, Yr3b, Yr4b, Yr-Su are respectively for 92.0%, 21.9% and 97.5%. CYR32 is firstly dominant race in the monitored 36 races, with the frequencies of 26.6%. While the second populated race is CYR31 with the frequencies of 16.9% And those races such as CYR21, CYR23, CYR25, CYR27, CYR28, CYR29, CYR31, are not the main races, and only with the frequency of 0.1%~1.7%. It’s notable that in 2010, there are 20 pathogenic strains are monitored to variety Guinong 22, Zhong 4, Chuanmaii 42, 92R137 and T. spelta album. At present, Guinong 22, 92R137 and T. spelta album are all immune to CYR31, CYR32 and CYR33, which are used as resistant resources in many breeding plans. The occurrence of these new strains might be a potential threat and should be paid more attention.

**Studies on viability of sclerotia collected from Sclerotinia stem rot infected soybean plants in Iowa during 1995–2010**

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Phytopathology 101:S83

Sclerotinia stem rot of soybean is caused by an ascomycetous fungus Sclerotinia sclerotiorum. In severe cases, this disease can cause up to 50% yield reduction in commercial fields. In developing management approaches of the disease, sclerotial viability plays an important role. Therefore, we studied viability of sclerotia collected during 1995–2010 from naturally infected soybean plants, northeast research and demonstration farm, Iowa and were stored in clear glass vials with screw caps at lab temperature. Viability of sclerotia was tested following germination test on PDA and anethocutis production on sterilized vermiculite (20 g vermiculite +10 ml DSW). In
germination test, 20 surface sterilized sclerotia from each year were placed on PDA (5 sclerotia per plate), a week after incubation at 21 ± 1°C sclerotia was evaluated for mycelium production, after sub-culturing of that mycelium evaluated for sclerotia production. Sclerotia from 2008 to 2010 showed >90% germination and reproduction of sclerotia, while sclerotia collected between 1995 and 2007 showed low or 0% germination. Similarly, 20 surface sterilized sclerotia from each year were placed on vermiculite (5 sclerotia per plate). Weekly examination for five months did not show any apothecia. If the sclerotia were collected from the white mold infested soil and were stored in soil, might have different inferences. This study may aid in development of management strategies of white mold.

Insecticidal activity of cantharidin against Plutella xylostella and its toxicological mechanism in Lepidopteran cells

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Stomach toxicity of cantharidin to 3rd larvae of Plutella xylostella and its effects on activities of glutathione-S-transferase (GSTs) and acetylcholine esterase (AChE) were tested in the laboratory. The results showed that the cantharidin had the stomach poisoning action on the Plutella xylostella larvae. At a concentration of 5 mg/mL, the corrected mortality of the larvae was 100% 72 h after cantharidin application. The LC₅₀ against the 3rd larvae were 515.58 µg/mL for 72 hours. In addition, At the dosage of 12.5 × 10⁻³ mg/mL of the Plutella xylostella larvae were treated by cantharidin, the highest GSTs activity was 2825.17 U·mg⁻¹·min⁻¹ at 12 h, while the highest AChE activities was 12.27 U·mg⁻¹·min⁻¹ at 4 h. While the GSTs and AChE activities had significantly decreased at 24 h. The outcomes suggested cantharidin can influence GSTs and AChE activities of Plutella xylostella larvae. DNA damage level of Spodoptera frugiperda cells (s-f9) after treated by cantharidin was detected by comet assay. After different concentrations of cantharidin incubation, tail DNA, tail length of cultured s-f9 increased, and head DNA decreased while the concentration of cantharidin increased. There were no differences compared with the control group. The results indicated that higher the dose of cantharidin, severer the DNA damage of Lepidopteran cell lines. DNA damage of cantharidin incubated s-f9 was of obvious dose-effect relation, which would indicate that DNA damage played a role in toxic effect mechanism.

Identifying genes differentially expressed during early interactions between the stem rot fungus (Sclerotium rolfsii) and peanut (Arachis hypogaea)

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Cultivated peanut is a major source of food and vegetable oil worldwide. Sclerotium rolfsii causes stem rot in peanut. The objective of my project is to identify genes in the fungal and peanut genomes that are differentially expressed during the early phase of the plant-pathogen interaction. By focusing on the early phase we expect to identify recognition events and cell signaling mechanisms. Four peanut cultivars varying from resistant to susceptible and one virulent fungal strain were selected. The fungal strain used is known to be virulent on peanut. Total RNA from infected stem and crown tissue was extracted, cDNA synthesized and 454 sequencing was performed. This generated 260390 sequences. Automated trimming and validation resulted in a dataset generated 225793 sequences. Comparisons of the sequences from each sample should highlight differential gene expression related to host plant resistance. Differentially expressed genes identified during bioinformatic analysis will be confirmed using qrt-PCR. Understanding the different genetic and biochemical pathways expressed early in the infection process will potentially provide new targets to disrupt the life cycle of S. rolfsii, stopping or slowing infection by this significant peanut disease-causing organism. Additionally, identification of pathogen responsive genes in peanut will add to the understanding of gene expression in peanut and could identify valuable sequences useful in potential future transgenic disease control strategies.

Comparative host response of grapefruit and alemow to narrow and broad host range strains of Xanthomonas citri subsp. citri

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Phytophthora ramorum research at the National Ornamentals Research Site at Dominican University of California


Phytopathology 101:S85

World-wide, Phytophthora ramorum has emerged as a quarantine-pathogen of significance, spurring emergency regulatory actions to address the spread of *P. ramorum* within ornamental nurseries and from infested nursery stock to native wildlands. After four years of collaborative efforts between California Department of Food and Agriculture, U.S. nursery industry, California Oak Mortality Task Force, National Plant Board and USDA, a site to perform research on *P. ramorum* under natural conditions was located in California. Funding via the U.S. Farm Bill (Section 10201) was secured and administered by the Center for Plant Health Science and Technology to develop the National Ornamentals Research Site at Dominican University of California (NORS-DUC). Situated in *P. ramorum*-quarantined Marin County, California, NORS-DUC was developed with safeguards to contain the pathogen and prevent spread to the environment. NORS-DUC has provided an unparalleled opportunity for researchers that are now conducting field studies to address the treatment of and prevention of *P. ramorum* in soil, the risk of asymptomatic infection in fungicide-treated plants, the effect of fungicides on inoculum production, biological control agents, and the affects of abiotic stress and ramorum blight in nursery ornamentals. While the current research on *P. ramorum* focused on inoculum production, biological control agents, and the affects of abiotic stress and ramorum blight in nursery ornamentals. While the current research on *P. ramorum* focuses on *P. ramorum* and will benefit the nursery and forestry industries, the data gathered will be applicable to the management of other pathogens.

Management of papaya mealybug, *Paracoccus marginatus* through biological control

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Phytopathology 101:S85

Papaya mealybug, *Paracoccus marginatus* has got introduced into Tamil Nadu during July, 2008 and subsequently spread to southern states of India. The pest infested more than 80 hosts and the damage varied from 5–100 per cent. Integrated Pest Management practices helped to contain the pest below ETL for a fortnight and again the infestation reappeared in 10–15 days which necessitated the farmers to go for repeated application of insecticides. Since no effective native parasitoids are available, three parasitoids viz., *Acerophagus papayae*, *Pseudlepptomastix mexicana* and *Anagyrus loeckii* were imported from Puerto Rico, U.S.A. *A. papayae* was mass multiplied in 57 research stations of Tamil Nadu Agricultural University located across Tamil Nadu. The parasitoids were released from October, 2010 onwards @100/village in the infested crops of papaya, cassava and mulberry. So far three hundred thousand parasitoids were released across the state. Observations on pre and post release data revealed that there was 80 – 99 per cent reduction of mealybug in papaya, cassava and mulberry. Multiplication rate of parasitoid was 10–15, 5–10 and 8–14 times in papaya, cassava and mulberry respectively. Due to the adoption of classical biological control the mealybug is under control in Tamil Nadu, India. This is one of the success example of classical biological control in Tamil Nadu, India in recent years.

Relationship between stink bugs and seed decay in Mississippi soybean production


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Phytopathology 101:S85

In recent years, soybean quality has become an important issue associated with Mississippi soybean production. Fungi, including *Phomopsis* spp. are major contributors to quality issues; however, other biotic and abiotic factors reduce seed quality. The stink bug is a major pest of soybean throughout the world. Stink bugs damage soybean by penetrating the pod wall with piercing-sucking mouth parts and extracting nutrients from the maturing seeds. However, depriving the seeds of vital nutrients is not the only risk to soybean. Damaged pod hulls likely result in the seeds being exposed to potentially pathogenic organisms that can further reduce yield and quality. The *Diaporthyphoma Phomopsis* complex is comprised of several fungi that cause yield and quality loss in soybean, with the primary pathogen being *F. longicolla*. To determine the relationship between stink bugs and seed decay in Mississippi soybean production; a survey of producer fields was conducted. Soybean seeds collected from 30 producer fields were divided into three categories including unblemished, damaged, and stink bug damaged. Seeds were surface disinfested and plated on acidified potato dextrose agar. The frequency of fungi was determined for each seed group. Significantly higher numbers of fungi were recovered from stink bug damaged seed compared to the other two groupings. The results indicate that stink bug damaged seeds contain a significantly higher percentage of fungi compared to unblemished seeds.

Limited effects of foliar insecticidal treatments on the spread of grapevine leafroll disease

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Phytopathology 101:S85

A field experiment was conducted at the experimental farm to investigate the effectiveness of foliar insecticide sprays on controlling the spread of grapevine leafroll disease (GLRD). The vineyard block has 20-year old Cabernet Sauvignon grapevines infected with GLRD. The symptomatic vines were tested positive for GLRaV-3 in one tube-single step RT-PCR using primers specific to a portion of the heat-shock protein-70 homolog (HSP70h). Within each row, all but one GLRaV-3-infected vines were removed and replanted with certified Cabernet Franc cuttings at approximately 5 and 10 feet away from each infected vine. After planting, foliar insecticide treatments were applied in a randomized block design of six replications. The treatments were (i) a neonicotinoid treatment at delayed dormant stage, (ii) a neonicotinoid treatment at delayed dormant stage plus a pyrethroid treatment in (iii) control. Petiole samples were collected in 2009 and 2010 seasons from individual vines of Cabernet Franc and extracts from these samples were tested for GLRaV-3 as described above. The results from both the seasons indicated that spread of the virus from Cabernet Sauvignon to Cabernet Franc vines could occur as soon as a few months after planting of new vines. Insecticide application appeared to have effectively limited the movement of the vector during the first year, but mealybugs were observed even on the treated vines during the second year of the season.

Multiple copies of genes encoding endoglucanase inhibitor proteins are harbored in an 85kb region of potato genome

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Phytopathology 101:S85

The XEGIPs (xylolog-specific endoglucanase inhibitor protein) and their closest homologues, the EDGPs (extracellular dermal glycoproteins) have been reported in various plants, principally Solanaceous ones. One function of XEGIP is limiting pathogen attack by interfering with pathogen family 12 endoglucanases. The XEGIP gene from tomato and potato was believed to be a single copy, however a cluster of nine similar genes has been found on a single region of potato chromosome, independent of the single XEGIP previously reported. A similar number of Petiole samples were collected in 2009 and 2010 seasons from individual vines of Cabernet Franc and extracts from these samples were tested for GLRaV-3 as described above. The results from both the seasons indicated that spread of the virus from Cabernet Sauvignon to Cabernet Franc vines could occur as soon as a few months after planting of new vines. Insecticide application appeared to have effectively limited the movement of the vector during the first year, but mealybugs were observed even on the treated vines during the second year of the season.

Pathogenicity of Coconut cadang-cadang viroid (CCCVd) variants on oil palm (*Elaeis guineensis* Jacq.) seedlings

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Phytopathology 101:S85

Coconut cadang-cadang viroid (CCCVd) that causes the Coconut cadang-cadang disease in coconut palms in the Philippines is associated with Orange spotting (OS) disease of oil palm. Hanold and Randles (1989) isolated CCCVd-like molecules from oil palm in the South Pacific and reported that *Phytophthora infestans* cause a yield reduction of 50% in single palm compared to healthy adjacent palm. Recent study by Vadamalai et al. (2006) confirmed that CCCVd variants were present in commercial oil palm plantation in Malaysia. Cloning and sequencing revealed that the CCCVd variants from oil palm oils has more than 90% sequence similarity with CCCVd<sub>sp</sub> from coconut. However mechanical transmission of the CCCVd variants to oil palm has yet been conducted. In this study, nucleic acid extracted from symptomatic palms was inoculated into oil palm seedlings. Successful transmission of CCCVd
variants in oil palm seedlings were observed when OS symptoms were expressed 6 months after inoculation. This was further proven with dot blot assay when the nucleic acid extract of the inoculated seedlings hybridized with CCCVd full-length complementary probe. Cloning and sequencing revealed that all variants from inoculated seedlings were 246 nt in length and has 97% sequence similarity with coconut CCCVd246. This study confirmed that CCCVd variants isolated from oil palm were pathogenic and replicating autonomously in its host.

Cherry leaf spot disease management in ornamental flowering cherry J. O. Joshua (1), M. T. MMBAGA (2), L. Mackasmiel (3)
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Phytopathology 101:S86

Cherry leaf spot (CLS) disease caused by a fungus Blumeriella jaapii is an important disease of sweet and sour cherries and other Prunus species; it has increasingly become a significant constraint in nursery production of flowering cherries causing great concern to growers in the Southeastern United States. While growers have attempted to control this disease with fungicides, efficacy of fungicide sprays has been poor because of poor timing of spray programs starting May. The objective of this study was to evaluate winter survival of the pathogen and assess the timing of infection establishment in mid-Tennessee to guide growers. In addition, previously inflected plants were maintained in greenhouse environment protected from airborne inoculum and assessed for CLS disease development. Air-borne ascospores trapped from previously inflected fields showed that infected leaf debris provided significant amounts of primary inoculum starting in early March with a peak in mid May. First CLS symptoms were observed in early April and fungicide spray program that started when petals start falling and new leaves start forming were highly effective in disease control. Development of cherry leaf spot symptoms in greenhouse plants showed that dormant buds constitute an important source of primary inoculum in Tennessee and the use of cuttings from infested trees may play a significant role in disease perpetuation.

Development of a Tobacco streak virus (TSV)-based gene silencing vector for soybean seed development S. JOSSEY (1), A. K. Singh (2), S. A. Ghahrial (2), L. L. Domier (3)
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Phytopathology 101:S86

Virus-based gene silencing systems are powerful tools for functional genomics that permit knockdown of expression of individual genes or closely related gene families. TSV shows recovery from initial symptoms and efficiency of silencing as key attributes and potential utility in developing soybean making it an excellent candidate for a virus-based silencing system for those tissues. TSV RNAs 1, 2, 3, and 4 were cloned into pHS740, a pUC-based plasmid vector, and pCASS-4RZ, an Agrobacterium tumefaciens-compatible binary vector. Both sets of clones were infectious in soybean and tobacco. A multicloning site was introduced into a truncated 2b gene of pHST40-RNA2 and the clone was stably seed transmitted in soybean. Magnesium chelatase (MgCh) gene fragments of 105 nt and 175 nt were inserted into the truncated 2b vector and were stable in systemic leaves of inoculated ‘Williams82’ plants, which exhibited pronounced leaf yellowing typical for silencing of MgCh mRNA. RNA 3 of the pCASS-4RZ clone was partitioned between two RNAs, one with only the movement protein (pCASS-R3Mp) and the other expressing only the coat protein (pCASS-R3Cp). Full-length green fluorescent protein (GFP) and magnesium desaturase (PDS) coding regions were inserted into pCASS-R3Mp and pCASS-R3Cp, respectively. Inoculated tobacco plants showed stable expression of GFP and photo-bleaching symptoms, which is consistent with silencing of PDS mRNA. The tissue specificity and persistence of silencing phenotypes are all being evaluated in soybean and tobacco.

Application of the 2-cyanoacetamide method for spectrophotometric assay of cellulase enzyme activity W. M. JURICK II (1), I. Vico (2), V. L. Gaskins (2), B. D. Whitaker (2), K. A. Peter (2), W. J. Janisziewicz (3), W. S. Conway (2)
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Phytopathology 101:S86

Cellulose is the most abundant form of carbon on the planet. Breakdown of cellulose in the plant cell wall is a means by which microbes gain ingress into their hosts. Cellulose degradation is also important for global carbon recycling and is the main substrate for the production of biofuels. The alkaline dinitrosalicylic acid (DNS) method is widely used to assay the enzymatic hydrolysis of cellulose, but is influenced by incubation conditions, and utilizes phenol. Therefore, we have developed a cellulase assay that is capable of detecting D-glucose using carboxymethylcellulose (CMC) as a model substrate. Our data show that this method is linear and sensitive as the DNS test in detecting fungal cellulase activity. Other factors that may affect the detection of cellulase activity such as: compatibility with commonly used buffer systems, varying buffer pH, and methods to terminate the enzyme catalyzed reaction, will be presented. Data from this study can directly be used to accurately and efficiently assay cellulase activity in a wide range of buffer systems at various pH’s without the use of potentially hazardous chemicals.

Nature of Ceratocystis smalleyi – Scolytus quadrispinosus interactions on stems of bitternut hickory with declining crowns J. JUZWIK (1), M. Banik (2)
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Phytopathology 101:S86

Rapid crown decline and mortality of bitternut hickory in north central and northeastern U.S.A. forests recently have been attributed to damage of host stems by the canker fungus, Ceratocystis smalleyi, and the hickory bark beetle (Hbb), Scolytus quadrispinosus. However, the nature of the interactions between these organisms in causing disease is unclear. Three trees, between 23 and 27 cm dia, with crown decline ratings of 40, 55 and 80% were debarked and found to have 178, 997, and 1,488 Hbb attacks on the main stem, respectively, in two Wisconsin forest stands. Of these attacks 24, 105, and 551 were associated with inner bark necrosis and discolored sapwood typical of Ceratocystis canker. Only 15 cankers had no evidence of beetle attack. Isolations from Hbb-associated cankers routinely yielded C. smalleyi. The fungus also was commonly isolated or detected via PCR and cloning on exoskeletons of Hbb captured during initial attack of hickory in late summer. However, C. smalleyi was not isolated from 120 Hbb emerged from stem sections of four trees with >55% crown decline, although 3 of 41 adults obtained by bark excavation prior to emergence did yield the fungus. In summary, Ceratocystis cankers are very frequently associated with Hbb attacks but evidence for dispersal of the pathogen from diseased trees by Hbb is lacking. The hypothesis that Hbb is the primary vector of the pathogen needs further study.

Sclerotinia sclerotiorum utilizes oxalic acid to hijack defenses and manipulate the host redox environment M. KABBAGE (1), B. Williams (1), H. Kim (1), M. B. Dickman (1)
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Phytopathology 101:S86

Sclerotinia sclerotiorum is a necrotrophic ascomycete fungus with an extremely broad host range. This pathogen produces the non-specific phytotoxin and key pathogenicity factor, oxalic acid (OA). Our recent work indicated that this fungus and more specifically OA, can induce apoptotic-like ROS induction, callose deposition, and restricted cell death. We used transmission electron microscopy (TEM) to show that the restrictive cell death observed upon A2 challenge, involves an autophagic response. These results indicate that S. sclerotiorum utilizes oxalic acid to hijack defenses and manipulate the host redox environment.

Siderophore loci in Agrobacterium vitis strain F25 are associated with its ability to provide biological control of grape crown gall S. KAEWNUM (1)
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Phytopathology 101:S86

Crown gall of grape, caused by Agrobacterium vitis is a limiting factor in grape production worldwide. A non-tumorigenic strain of A. vitis, F25, is able to prevent crown gall on grape when applied to wounds prior to the pathogen. F25 was sequenced and mutations were made in biological control candidate genes and mutants tested for activity on grapevines. Three siderophore loci were detected in F25, one that is unique among sequenced Agrobacterium
species. Knockouts within this cluster and in another cluster resulted in biological control-negative phenotypes. The knockouts did not affect the ability of F25 to cause necrosis of grape explants or induce a hypersensitive response on non-host plants. The effect of F25 and mutants on populations of the pathogen in wounded grape tissues was also evaluated.

Influence of fungicide timing and post application irrigation on dollar spot severity

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Phytopathology 101:S87

Dollar spot, caused by the pathogen Sclerotinia homoeocarpa F.T. Bennett, is a common disease of turf course turf. Our study was conducted to compare early versus traditional preventive applications of different fungicides and the influence of post application irrigation on disease suppression. A total of seven evaluations were conducted between 2008 and 2010 in Connecticut and Pennsylvania. All studies were designed as a 2 x 2 x 4 factorial and arranged as a randomized complete block with 4 replications. The main treatments included timing (mid-April or mid to late May), irrigation (none or 2.5 mm), and fungicide (none, propiconazole, boscalid or vinclozolin). All treatments decreased dollar spot when compared to the untreated control plots, but few differences were observed among the main effects. Of 50 rating dates assessed across all studies, dollar spot was reduced on only 5 and 4 dates in plots treated at early or traditional timings, respectively. Irrigation was only significant on 3 of 50 rating dates and in all cases, the application of post application irrigation resulted in an increase in dollar spot severity when compared to plots receiving no irrigation after application. Results of this study indicate that while early season fungicide applications may suppress dollar spot infection centers, they may offer little benefit over properly timed preventive fungicide applications.

Correlation of environmental and edaphic factors to the isolation frequency of Rhizoctonia and Chrysorhiza from seashore paspalum

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Phytopathology 101:S87

Hymenomycete genera include the anamorphs Rhizoctonia and Chrysorhiza. Species of fungi in these genera have similar anamorphic characteristics, and their respective teleomorphic structures rarely are observed in nature. Comparison of conserved DNA sequences such as iDNA that includes internal transcribed spacer (ITS) 1, the 5.8S ribosomal subunit, and ITS2 can help identify some of these fungi to species. 74 isolates within this class of fungi were recovered from turf tissue samples taken from eight different Florida golf courses. Soil and canopy temperatures, soil electrical conductivity (EC) as a measurement of total soluble salts and soil pH data were taken at each sample date and each location. The species, variety and/or anastomosis group of the hymenomycete isolates were identified. Soil temperature had a significant effect on isolation of R. solani AG 2-1LP (P = 0.0001) and was negatively correlated (Pearson correlation coefficient, r = -0.61). Soil temperature did not significantly affect isolation frequency of any Chrysorhiza sp. Soluble salt concentration was positively correlated (r = 0.33) with isolation frequency of all hymenomycetes (P = 0.009). Rhizoctonia solani AG 2-1LP recovered was more likely to occur during periods of higher soil temperatures. Chrysorhiza spp. were isolated over a wider range of temperatures. Increasing levels of soil salinity were observed to correlate to higher frequencies of isolation of Rhizoctonia and Chrysorhiza fungi.

Yield loss in spring wheat due to disease caused by Xanthomonas campestris pv. translucens

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Phytopathology 101:S87

Occurrence of bacterial leaf streak and black chalk caused by Xanthomonas campestris pv. translucens has increased in the Northern Great Plains in recent years, however the potential for crop loss has not been studied. This study was conducted in Brookings, SD with an objective to estimate the losses due to the disease in spring wheat. Five spring wheat genotypes with various levels of resistance to leaf streak were evaluated under inoculated and uninoculated conditions. A virulent isolate XcStSD17 was inoculated at tillering stage to induce disease in inoculated plots. Disease development was faster and more severe in inoculated plots though some disease was also present in uninoculated plots. Grain yield was significantly different between inoculated and uninoculated plots in all the genotypes. Inoculated plots had 12 to 32% lower yields compared to uninoculated plots. Yield difference between inoculated and uninoculated plots was higher in susceptible genotypes SD3948 (32% loss) and Russ (29% loss) while as moderately resistant SD1418 had the lowest yield reduction (12%) among the tested genotypes. Test weight was poor in all genotypes however inoculated had lower test weights than uninoculated plots, with up to 7% reduction observed. These findings showed that significant yield loss due to the disease can be expected if the conditions are conducive. Additionally, this study highlighted the potential importance of the disease in a breeding program since no effective in-season control is available.

Red potato cultivar (Solanum tuberosum L.) susceptibility to the root-knot nematode Meloidogyne incognita

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Phytopathology 101:S87

Yield loss in some potato cultivars (Solanum tuberosum L.) by Meloidogyne spp. (root knot nematodes) may reach 80% in tropical and subtropical areas. Susceptibility of red potato cultivars to M. incognita is generally unknown and requires investigation to appropriately manage yield losses. In a greenhouse, four population densities of M. incognita were evaluated on eight red skinned potato cultivars. Tuber weight (TW) and nematode reproduction factor (RF) (RF = P/Fp) were used to indicate tolerance and resistance. TW of cultivars Desiree, Pink Pearl and Mountain Rose were above the overall mean by 36%, 27%, and 9%, respectively. TW was less than overall mean for Durango, Red Thumb, All Red, Colorado Rose and Rote Ersting (20%, 20%, 11%, 11%, and 9%, respectively). A ranking of RF showed that Desiree, Pink Pearl and Red Thumb were consistently among the cultivars supporting the greatest nematode reproduction regardless of Pr (RF < 1). Durango, Rote Ersting and Mountain Rose were consistently among the most resistant cultivars (RF < 1). Desiree and Pink Pearl placed in a tolerant-susceptible category, whereas; Red Thumb was intolerant-resistant. Intolerant-susceptible and all other cultivars were intolerant-resistant. This information on red skinned potato cultivars will be useful for breeders and growers in selecting cultivars tolerant and resistant to M. incognita, mitigating yield loss.

Viruses associated with yellow vein and vein enation disease of citrus

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Phytopathology 101:S87

Citrus yellow vein (YV) and vein enation (VE) are graft-transmissible diseases of unknown etiology. YV symptoms include yellowing of the main veins and adjacent petiole areas in almost all citrus species whereas synergism between YV and other graft-transmissible citrus diseases such as VE significantly enhance symptom expression. VE symptoms include vein enation and gall formation in susceptible hosts, such as Mexican lime (ML) and rough lemon with reports indicating that a luteovirus may be associated with VE. ML seedlings were graft inoculated with blind buds from YV and VE symptomatic plants and developed typical YV and VE symptoms eight weeks post inoculation. High molecular weight double stranded RNAs were isolated from the inoculated MLs, but not from the un-inoculated controls. After shotgun cloning and sequencing, several sequenced clones showed high degree of homology with umbraviruses and luteoviruses. The YV luteovirus species was also identified in clones obtained from the VE inoculated plants. It is proposed that the luteovirus is a major component of both YV and VE and that VE evolves to YV as a result of the synergism between the umbra- and the luteovirus.

Performance of recombinant inbred line populations segregating for Fusarium virguliforme resistance in soybean (Glycine max (L.) Merr.)

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Phytopathology 101:S87

Sudden death syndrome (SDS) caused by Fusarium virguliforme is a devastating disease in soybean (Glycine max (L.) Merr.) that causes yield losses up to 70% depending on the developmental stage that plants become infected. Characterization of resistance is greatly significant for genetics and breeding studies. Two populations were developed for this study by crossing two lines from Southern Illinois germplasm collection, LS90-1920 x Spencer, and LS97-1610 x Spencer) were evaluated for two years (2009 and 2010) at three diverse locations (Carbondale, New Haven and Valmeyer IL) in Southern Illinois. Population statistics, genotype x environment interaction, and broad-sense heritability were used to reveal any major resistance genes. Genetic correlation coefficients of SDS resistance with important agronomic traits such as lodging, pubescence, growth habit, and plant height were also calculated. The information from this study will be helpful to breeders in developing mapping populations and enforcing selection practices.
Anthracnose of sweet pepper caused by Colletotrichum simmondsii found in Iran

In September 2009, severe fruit rot of sweet pepper were found in Hyogo prefecture, Japan. The symptoms were circular to elliptoid and sunken spots of 5–30 mm diameter, with concentric rings of gray-brown to black, and abundant orange masses of conidia. Stems and leaves sometimes infected of which symptom closely resembled to "Cercospora leaf spot". Two isolates obtained from the diseased sweet pepper were examined in an inoculation test by attaching mycelial disks to artificially wounded fruits. The symptoms were reproduced and the same fungus was re-isolated. The two isolates formed neither sclerotia nor seta. Conidia were subcylindrical, attenuated and blunt pointed ends without gullet. The conidia were 11.3–25.7 micrometers length and 2.7–5.2 micrometers breadth. L/B = 3.9. Appressoria were smooth, grayish brown obovoid to ellipsoid and 4–17 micrometers length and 3.2–6.8 micrometers breadth. Colonies on PDA that are gray cottony and in reverse pale gray to pale orange sometimes with dark flecking. The morphological and cultural characteristics were in accordance with Colletotrichum simmondsii. rDNA ITS and beta-tubulin-2 sequences of the isolates were identical and had high similarity to those of C. simmondsii. These isolates were also virulent to tomato, string bean, and strawberry.

Genetic diversity of Potato virus Y\(^\*\) and origin of recombinant PVY strains
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The ordinary strain of Potato virus Y (PVY), PVY\(^*\), induces necrosis and severe stunting in potato cultivars carrying the Ny gene. A novel sub-strain of PVY\(^\text{O}\), PVY\(^{\text{O-OS}}\), has been found spreading in the U.S.A. molecular study of PVY\(^\text{O}\) and PVY\(^{\text{O-OS}}\) isolates from a North American collection of PVY was conducted through whole genome sequencing and phylogenetic analysis. Forty-four PVY\(^\text{O}\) isolates were sequenced, including 31 from the PVY\(^\text{O-OS}\) group. PVY\(^\text{O-OS}\) isolates formed a separate, novel evolutionary lineage of PVY from potato. To shed light on the origin of the three most common PVY recombinants, a more detailed phylogenetic analysis of a sequence fragment, nt 2,406–2,821, that is present in all recombinant and non-recombinant PVY\(^\text{O}\) genomes was conducted. The analysis revealed that PVY\(^\text{O}\) and PVY\(^{\text{O-OS}}\) recombinants arose from two PVY\(^\text{O}\) lineages, while the PVY\(^{\text{NNT}}\) recombinant acquired its PVY\(^\text{O}\) segment from the same lineage as PVY\(^{\text{NO}}\). These data suggest that PVY\(^{\text{NO}}\) and PVY\(^{\text{NWi}}\) recombinants originated from two separate recombinant involving two different PVY\(^\text{O}\) parental genomes, while the PVY\(^{\text{NNT}}\) recombinants likely originated from the PVY\(^{\text{NNT}}\) genome via additional recombinant events.

Genetic diversity of Fusarium verticillioides isolated from Corn in Iran
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Forty-four PVY\(^\text{O}\) isolates were sequenced, including 31 from the PVY\(^\text{O-OS}\) group. A phylogenetic analysis of the PVY\(^\text{O}\) isolates revealed that the PVYO, PVYO-O5, has been found spreading in the U.S.A. molecular study of PVY\(^\text{O}\) isolates from Sweet corn in different countries. The isolates were identified morphologically and by attaching mycelial disks to artificially wounded fruits. Two isolates obtained from the diseased sweet pepper were examined in an inoculation test by attaching mycelial disks to artificially wounded fruits. The symptoms were reproduced and the same fungus was re-isolated. The two isolates formed neither sclerotia nor seta. Conidia were subcylindrical, attenuated and blunt pointed ends without gullet. The conidia were 11.3–25.7 micrometers length and 2.7–5.2 micrometers breadth. L/B = 3.9. Appressoria were smooth, grayish brown obovoid to ellipsoid and 4–17 micrometers length and 3.2–6.8 micrometers breadth. Colonies on PDA that are gray cottony and in reverse pale gray to pale orange sometimes with dark flecking. The morphological and cultural characteristics were in accordance with Colletotrichum simmondsii. rDNA ITS and beta-tubulin-2 sequences of the isolates were identical and had high similarity to those of C. simmondsii. These isolates were also virulent to tomato, string bean, and strawberry.

Functional characterization of the PidS/PidR two-component regulatory system of Burkholderia glumae
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Phytopathology 101:S88

Burkholderia glumae is the major causative agent of bacterial panicle blight of rice, which frequently causes significant yield losses in many rice-producing countries. Environmental factors, especially high temperature and humidity, are thought to be closely related to outbreaks of this disease. Little is known, however, about the signaling mechanism that perceives and transduces environmental signals that induce expression of virulence genes in this pathogen. In this study, we sequenced the genomes of two different B. glumae strains producing different pigments in certain nutritional conditions, including CPG medium. Furthermore, the production of these pigments is abolished by mutation of the genes encoding a two-component regulatory system (TCRS) comprised of pidS and pidR that encode a sensor histidine kinase and a response regulator, respectively. Remarkably, pidS and pidR mutants failed to elicit an hypersensitive response on rice leaves and showed attenuated virulence in rice. In addition, these mutants produced reduced amounts of the phytotoxin, toxoflavin, a major virulence factor of B. glumae. This is the first report of a TCRS involved in the pathogenesis of B. glumae. To better understand the regulatory function of the PidS/PidR TCRS, expression patterns of virulence genes and other regulatory genes of this pathogen in pidS and pidR mutant backgrounds are currently being investigated with a gas reporter system and a quantitative-PCR technique.

Comparative genomics of a lucerne and non-lucerne isolate of Verticillium albo-atrum
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Phytopathology 101:S88

Verticillium albo-atrum is a fungal pathogen that causes a wilt disease of trees, herbaceous plants, and agricultural crops. Because of its significance as a plant pathogen, both the mitochondrial (29.49 Kb) and nuclear genome (32.83 Mb) of V. albo-atrum isolate VaMs.102 from lucerne (alfalfa) were sequenced by Broad Institute in 2008. However, previous studies based on nuclear markers clearly revealed two homologous populations (lucerne and non-lucerne) within V. albo-atrum. Without
sequenced genomes for both intra-specific populations, differences in pathogenic adaptation and allelic variability in virulence-associated genes remain incomplete. Utilizing both 454 and Illumina sequencing technology, we generated a combined 14X genome assembly of V. albo-atrum PSU140 from Ailanthus for both mapping against the reference (VaMs.102) and de novo assembly. V. albo-atrum PSU140 was isolated from diseased Ailanthus altissima in mixed oak forests in south-central Pennsylvania and has been characterized with regard to efficacy and host specificity. Here we show the 32.4-megabase (Mb) genome assembly of PSU140 in comparison with the 32.83 Mb draft genome of VaMs.102. Pairwise comparisons of total mappable contigs between VaMs.102 and PSU140 reveals sequence similarity of 96.51%. Through these comparisons, we can begin to elucidate the molecular mechanisms that underlie pathogenicity, differentiation, and host-adapted virulence in PSU140.

Risk analysis for Verticillium albo-atrum isolate PSU 140, causal agent of Verticillium wilt of tree-of-heaven (Ailanthus altissima)

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Phytopathology 101:S89

Unprecedented wilt and mortality of the invasive tree-of-heaven (Ailanthus altissima) by Verticillium albo-atrum is currently epidemic in south-central Pennsylvania. Because V. albo-atrum causes wilt diseases of trees, herbaceous plants, and agricultural crops, >70 species were stem-inoculated with isolate PSU140 in the field and greenhouse. The following species exhibited wilt and vascular discoloration following inoculation: Ailanthus, Amur corktree, autumn olive, blackberry, black locust, corkwood, cymosine, devil’s walkingstick, elderberry, honey locust, Japanese barberry, Japanese maple, catalpa, Norway maple, poison-ivy, redbud, sassafras, staghorn sumac, striped maple, and tree-of-paradise. Of these, only six species had >10% mortality following wilt: Ailanthus, blackberry, poison-ivy, redbud, striped maple, and sumac. Furthermore, natural spread of V. albo-atrum within diseased Ailanthus stands was observed only for Ailanthus (100%), devil’s walkingstick (22%), and striped maple (4%). Vascular discoloration following inoculation, but without wilt or mortality, was observed on >20 species. Although artificial inoculations provide an evaluation of potential damage to non-target hosts, the low incidence of disease and mortality of these non-target hosts among inoculated Ailanthus offer support that PSU140 may be host adapted. Pending the outcome of host-range and molecular studies, V. albo-atrum should be considered as a potential biocontrol for the invasive tree-of-heaven.

Suppression of Fusarium spp. in tissue culture (TC) banana established in field soils inoculated with commercial biological products

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Phytopathology 101:S89

Fusarium oxysporum sp. cubense threatens the survival of TC Gros michel banana worldwide. Control by fungicides has failed with breeding rather than control of pathogen preferred. A study funded by BMGF was conducted by CIAT-Tropical Soil Biology and fertility institute to evaluate commercial biological and chemical products for use in Africa. A complete randomized design was used. Inoculation with 4 isolates of Fusarium spp. on verticils, cutric nitsolos and humic nitsolos from banana growing regions in Kenya. Fusarium spp. were isolated using Peptone Pentachloronitrobenzeneg ag. Identification manual for Fusarium by Burgess using cultural and microscopic characteristics distinguished isolates as Fusarium oxysporum. The isolates were white and pink in vertisol, white in cutric nitsol and purple with white tint and white in humic nitisol. Colony forming units (CFU) were significantly (p < 0.05) different. The CFU before inoculation was 8.0 × 102 for cutric nitisol, and vertisol and 2.5 × 102 in humic nitisol. Rhizatech reduced CFU in cutric nitisol and humic nitisol by 87.5% and 36% respectively. Ecot treatment reduced CFU in vertisol and humic nitisol by 12.5% and 44% respectively. Response to products depends on soil type and there is potential in use of products to suppress disease.

Controlling gummy stem blight in the greenhouse on watermelon seedlings grafted onto cucurbit rootstocks

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Phytopathology 101:S89

Interspecific hybrid squash (Cucurbita moschata x C. maxima) and bottle gourd (Lagenaria siceraria) are used as rootstocks for grafting seedless watermelon. Both rootstocks and watermelons are susceptible to gummy stem blight, caused by Didymella bryoniae, especially during healing, when grafted seedlings are held at high relative humidity or misted for 1 week while the vascular bundles of rootstocks and scions connect. The objective of this study was to control gummy stem blight on grafted seedlings with fungicides. To assess phytotoxity, rootstock seedlings were sprayed twice at weekly intervals with labeled rates of fungicides. Four of 9 fungicides injured 3–35% of the surface area of cotyledons on one or both rootstock species, and difenoconazole also stunt Cucurbita. Five fungicides were applied to ‘SS 7187H’ seedless watermelon, ‘Strong Tosa’ hybrid squash, and ‘Emphasis’ bottle gourd. The next day scions and rootstocks treated with the same fungicide were grafted together and placed in a humidity chamber. One day later grafted seedlings were inoculated and then held for 6 more days. All fungicides reduced incidence and severity of gummy stem blight compared with the water control (94% incidence, 9.7% severity). Difenoconazole and cyprodinil (<13% incidence, ≤0.1% severity) were more effective than mancozeb or cyprodinil+fluoxonil, which were more effective than thiophanate-methyl (P = 0.01).

Screening for powdery mildew resistance in ‘Ohelo berry germplasm in Hawaii

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Phytopathology 101:S89

‘Ohelo, Vaccinium reticulatum (Smith), is an endemic Hawaiian shrub, less than 1 m (3.3 ft) tall, and grows between 640 and 3700 m (2,100 to 12,100 ft) elevation on disturbed volcanic sites on the islands of Maui and Hawaii. Concerns have arisen about human impacts to the environment during the wild gathering of fruits that include spreading of exotic weeds, damaging native vegetation, and reducing a food source of the endemic nene goose, Banta sandwicensis (Vigor). As an alternative to wild harvest, ‘Ohelo cultivars for small-scale cultivation as ornamentals and for edible berries in Hawaii were identified, evaluated and selected. The main disease pressure that may limit berry production and ornamental qualities is from powdery mildew. Numerous disease resistance screens of diverse ‘Ohelo berry germplasm were conducted to identify powdery mildew resistance. Controlled inoculations were made onto leaf discs, detached leaves and potted seedlings. The results were compared with natural epidemics in two locations on Hawaii Island, Mealani and Lalamilo, approximately 55 miles north of Hilo. There was a good correlation of ratings between the field and potted plant ratings. No accession rated in all four screens was immune from infection. However, one cultivar (‘Kilauea’) was consistently rated as tolerant across three independent studies. The results emphasize the importance of uniform testing in multiple environments using the most appropriate host material available.

Leaf blight and stem canker of Mangosteen in Hawaii

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Phytopathology 101:S89

Mangosteen (Garcinia mangostana Linn.) is a tropical evergreen tree that produces one of the most desired tropical fruits, sometimes referred to as the “Queen of Fruit”. Mangosteen has the potential to occupy a rapidly expanding niche market in Hawaii. The aim of this study was to identify the causative agent of the leaf spots and stem cankers in Mangosteen trees ranging in age from newly planted to 6+ years old. Symptoms were observed in a Mangosteen orchard on the Hamakua Coast of the Island of Hawaii, approximately 6 miles north of Hilo. Single foliar and stem canker lesions from 27 plants were confirmed as Pestalotiopsis sp. and stem cankers resulted in pure cultures of the fungus. The fungus was identified as Pestalotiopsis sp. based on morphological characteristics and molecular analysis. Pathogenicity tests with the isolated fungus showed identical leaf symptoms on 3 year old seedlings growing in a hoophouse. Pestalotiopsis leaf blight has already been reported in other countries growing Mangosteen. However, this is the first report of Pestalotiopsis leaf blight and stem canker on Mangosteen in Hawaii.

Comparing foliar and drench application of azoxystrobin for controlling Rhizoctonia root rot of sugar beet

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Phytopathology 101:S89

Rhizoctonia crown and root rot caused by Rhizoctonia solani Kühn is the most important sugar beet disease for growers in Minnesota and North Dakota. Crown rot was common with conventional varieties since cultivation for weed control led to crown inoculation. Root rot is more common with transgenic varieties which are on 95% of the acreage. Foliar application of azoxystrobin before infection occurs will control the disease. The objective of this study was to evaluate and compare the effect of foliar and hypocotyl drench applications of azoxystrobin for controlling Rhizoctonia root rot caused by R. solani AG 2-2 IIIB. Treatments included a non-inoculated check; an
Potential of *Paecilomyces lilacinus* strain 251 to control the root-knot nematode *Meloidogyne enterolobii*, a new quarantine species for the EPPO region

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Phytopathology 101:S90

*Meloidogyne enterolobii* (syn. *M. majaguentensis*) was recently added to the EPPO list as an A2 quarantine organism. This species is causing severe damage on vegetables such as tomato and is particularly difficult to manage as it overcomes resistance against tropical root-knot nematodes. As organic farmers have no options to control *M. enterolobii*, *P. lilacinus* strain 251 (PL251) was tested in the water dispersible granule (Meloco WG) and a new water dispersible powder formulation (WP) in on-farm and semi-commercial yield trials, and under controlled environmental conditions. All studies demonstrated a high biocontrol efficacy of PL251 independent from the type of formulation used. Furthermore, the use of a surfactant or soy meal and sugar as additional food supplements affected the efficacy of PL251. Conversely to previous studies with *M. incognita*, stronger reduction of nematode damage by PL251 was observed at higher inoculum levels. Furthermore, high egg parasitism rates indicated an increased rhizosphere competence of PL251 which was never observed before. However, sufficient biocontrol efficacy still requires a pre-planting soil treatment to reduce initial nematode inoculum. It was demonstrated that the novel WP formulation of PL251 was equal or superior in its efficacy to control *M. enterolobii* on tomato when compared to the WG type formulation which, in combination with a 10-fold higher concentration and increased shelf-life, makes it a viable option for control of *M. enterolobii*.

QBOL - Barcoding as a new tool for identification of quarantine nematodes and their close relatives

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Phytopathology 101:S90

Identification of quarantine plant pests needs to fast and be accurate to enable timely plant protection measures. False diagnostics could cause serious financial losses to the economy and producers. Genetically engineered organisms, that is a reliable alternative to the classical identification generally based on morphological features requiring expert taxonomic skills. Genetic diagnostics through the use of DNA-barcodes, stretches of DNA that contain taxon-specific information, can be performed by any skilled lab-worker. The European Union 7th Framework project QBOL. “Development of a new diagnostic framework to support of plant health” aims to establish DNA-barcodes for all European quarantine organisms and their close relatives, including plant parasitic nematodes. For quarantine nematodes, several gene regions such as COI, COII, SSU, LSU and RNA polymerase subunit II are being evaluated for their barcoding potential. The results and protocols will be made available through a database, Q-bank, freely accessible to all interested users. For each group of quarantine organisms, a consortium of curators will ensure that data incorporated into Q-bank are confirmed for correctness and linked to specimen in reference collections.

Identification of the tropical root-knot nematode species *Meloidogyne incognita*, *M.arenaria* and *M. javanica* by a multiplex PCR protocol

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Phytopathology 101:S90

*Meloidogyne incognita*, *M. javanica* and *M. arenaria* are considered to be the economically most important root-knot nematode species due to a wide host range and high damage potential. Next to the detection of quarantine root-knot nematodes, identification of the morphologically similar tropical species is needed in routine testing. Therefore, a reliable multiplex PCR protocol was developed for rapid identification of *M. incognita*, *M. javanica* and *M. arenaria*. To guarantee a high specificity and reproducibility, primers which had been routinely used in our lab and produced reliable results in routine diagnostics were the basis for a multiplex PCR protocol. The SCAR primers Mjav/Fjav and Far/Rar produce species specific products of 720 bp and 420 bp for *M. javanica* and *M. arenaria*, respectively. A complementary primer for *M. incognita* was developed based on the 399 bp product of the SCAR primers inc-K14-F/R. Following sequencing of the amplicon, primers M2f4/M2R1 were designed to produce a product of 300 bp. This primer combination produced reliable results in multiplex PCR assays with 14 different populations from 5 countries. No cross reaction was found with *M. hapla*, *M. fallax*, *M. chitwoodi*, *M. enterolobii* and *M. ethiopica*. Furthermore, the amplified species specific products allow separation by high-resolution capillary electrophoresis and might be used in high-resolution-melting-curve analysis assays.

Severity risk spatial model for *Phytophthora* diseases in woody ornamental nurseries in southern Middle Tennessee

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Phytopathology 101:S90

Middle Tennessee has the largest concentrations of nurseries in the state. Many of the ornamentals trees and shrubs are highly susceptible to a variety of *Phytophthora* species. Disease severity risk map was calculated from a risk model based on the parameters that can be directly correlated to *Phytophthora* disease occurrence, pathogen reproduction and spread. These parameters include availability of susceptible host species, soil drainage, proximity to roads, and integrated moisture index (IMI). Technologies such as Global Positioning System (GPS), geospatial information systems (GIS), and remote sensing have been utilized to create a severity risk map for *Phytophthora*. Maps of each environmental parameter were created and overlaid to show the cumulative severity risk to *Phytophthora*. A final map was categorized into six risk levels of no risk, very low risk, low risk, moderate risk, high risk, and very high risk; nurseries were then added using GPS and remote sensing. A buffer area was applied to the GIS to surround each nursery. The percent of each severity risk level was calculated for each buffer region. Nurseries with the highest risk categories were at the highest risk for *Phytophthora* diseases. To validate this model, a survey for *Phytophthora* occurrences was done in nine counties. The severity risk model developed in this project will allow growers to predict *Phytophthora* risks in their areas for disease management preparedness.

First report of bacterial leaf spot on milk vetch (*Astragalus sinicus*) caused by *Pseudomonas viridiflava* in Korea

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Phytopathology 101:S90

Bacterial leaf spot disease occurred on milk vetch (*Astragalus sinicus*) grown in ornamental nurseries in southern Middle Tennessee. Bacterial leaf spot is caused by *P. viridiflava*. Artificial inoculation were essentially identical with those in the field. The bacteria isolated from symptomless milk vetch leaves were tested for pathogenicity to *A. sinicus*. In Korea, the bacteria were grown on TSA for 48 hr at 25°C. Pathogenicity of the bacteria was confirmed on milk vetch leaves with needle inoculation of bacterial suspension containing 10⁶ CFU/ml in sterile distilled water. Sterile distilled water was used as control. Inoculated plants were placed in a humid chamber with 100% relative humidity at 20°C for 14 days. Symptoms were assessed about 14 days after inoculation. Bacterial leaf spot symptoms of milk vetch plants produced by the bacteria were essentially identical with those in the field. The bacteria was reisolated from those lesions. Bacteria isolates causing leaf spot were detected and subsequently identified as *P. viridiflava* using the Biolog system and 16S rDNA phylogenetic analysis. This is the first report of bacterial spot of milk vetch caused by *P. viridiflava* in Korea.

Multiple resistance phenotypes of *Botrytis cinerea* in apple orchards and effects on control of gray mold in stored apples with postharvest fungicides

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Phytopathology 101:S90

*Botrytis cinerea* is the cause of gray mold in stored apples. To investigate fungicide resistant phenotypes of *B. cinerea* in apple orchards and their impacts on gray mold control in stored apples, isolates of *B. cinerea* were obtained from symptomless fruit from orchards where methyl benzimidazole carbamates (MBCs); cyprodinil, an anilinopyrimidine (AP); quinone outside inhibitors (QoIs); and boscalid, a succinate dehydrogenase inhibitor (SDHI)
had been used. All isolates were tested for resistance to these fungicides. Of the 219 isolates tested, 5.5% were resistant to all 4 fungicide classes; 12.8% were resistant to MBC and SDHI; 0.5% were resistant to QoI and SDHI; 0.5% were resistant to QoI; and 80.8% were sensitive to all 4 fungicide classes. Effectiveness of postharvest fungicides thiabendazole (TBZ), pyrimethanil (PYR), and fluoxonil (FLU) for control of gray mold on apple fruit incited by different phenotypes was evaluated. TBZ and PYR failed to control gray mold incited by TBZ- and PYR-resistant isolates, respectively; while FLU effectively controlled both quadraple- and triple-resistant isolates. All three postharvest fungicides effectively controlled gray mold caused by QoI- and SDHI-resistant isolates. The results indicated that multiple resistance of B. cinerea has developed in the orchards and that strategies for resistance management should be implemented to avoid the failure of gray mold control in stored apples with postharvest fungicides.

Competitive interactions between the biocontrol fungus Trichoderma harzianum and Fusarium solani f. sp. pisi in soil T. Kim (1), G. R. KNUDSEN (1) (1) University of Idaho, Moscow, ID, U.S.A. Phytopathology 101:S91

Competitive interactions were evaluated between the potential biocontrol fungus Trichoderma harzianum ThzID1-M3, as well as other indigenous Trichoderma spp. in soil, and the plant pathogen Fusarium solani f. sp. pisi (Fsp), both in soil and in the rhizosphere. Alginate pellets containing ThzID1-M3 (a green fluorescent protein-expressing recombinant strain), along with Fsp (1 × 10^7 conidia/g soil), were added to non-sterile slit loam soil into which pea seeds were then planted. Treatments were either ThzID1-M3 or Fsp alone, both organisms together, or neither organism added. Using quantitative real-time PCR, Fsp, ThzID1-M3 and other (indigenous) Trichoderma spp. were quantified in soil and on pea roots over a 21-day period. Addition of ThzID1-M3 to soil significantly (P < 0.05) reduced Fsp biomass in soil, and also reduced pea root colonization by Fsp. Addition of Fsp resulted in significantly lower biomass of both ThzID1-M3 and other Trichoderma spp. These results suggest that competition between Trichoderma and Fusarium solani f. sp. pisi negatively affects the establishment of both organisms in soil.

Comparative analyses of Korean isolates of Cucumber mosaic virus M. KIM (1), H. Kwak (1), S. Ko (2), S. Lee (1), J. Kim (1), K. Park (1), K. Kim (3), B. Cha (4), H. Choi (1) (1) National Institute of Agricultural Science, RDA, Suwon, SOUTH KOREA; (2) Jeonnam Agricultural Research and Extension Services, Naju, SOUTH KOREA; (3) Seoul National University, Seoul, SOUTH KOREA; (4) Chungbuk National University, Cheongju, SOUTH KOREA Phytopathology 101:S91

Cucumber mosaic virus (CMV) occurs naturally worldwide and has the broadest host range of any known virus. We have used twelve isolates of CMV; each two isolates from tomato and Virginia pepperweeds were selected, and each one isolate from cucumber, zucchini, red pepper, tobacco, radish, bean, angelica, Phasoleus ulmarius and adzuki bean were also used in Korea. Those isolates were investigated and classified by using the selective differential hosts from indicator host species and RNA analyses of CMV genomic RNAs 1, 2 and 3. Relationships of twelve isolates of CMV were also compared using pathogenicity on host plants, cytopathological alterations and phylogenetic analyses. To determine the pathogenicity of virus isolates and the symptoms, all the host plants were used. The same families were not found to be equally susceptible to CMV isolates of the same hosts. Phylogenetic analyses of the RNAs 2 and 3 ORFs, twelve CMV isolates could be clearly divided into three clades that correspond to subgroups IA, IB and IC. However, according to the pattern of the nucleotide sequences of 1a, CMV isolates of subgroup I could be divided into three clades (IA, IB and IC). For relationship of cytopathological alterations and CMV genome RNAs, tissues and cells were observed after inoculations of twelve CMV isolates by light and electron microscopy. No specific tissue and cell was observed by light microscopy, but virus particles and inclusion bodies could be easily found in cuticle, epidermis, parenchyma, collenchyma and vacuole. Biological and molecular characterization of Ribgrass mosaic tobamovirus infecting Rehmannia glutinosa M. KIM (1), H. Kwak (1), D. Lee (1), S. Ko (2), S. Lee (1), J. Kim (1), K. Park (1), B. Cha (3), H. Choi (1) (1) National Academy of Agricultural Science, RDA, Suwon, SOUTH KOREA; (2) Jeonnam Agricultural Research and Extension Services, Naju, SOUTH KOREA; (3) Chungbuk National University, Cheongju, SOUTH KOREA Phytopathology 101:S91

Rehmannia glutinosa is a member of the Scrophulariaceae family and is an important herbaceous medicinal plant in Korea. A virus causing symptoms of mosaic, stunt and malformation on Rehmannia glutinosa occurred around Hwasun area in Korea. Virus diseased plants were analyzed RT-PCR and electron microscopy. The sample was infected Broad bean wilt virus (BBWV2), Tobamovirus and other unidentified filamentous virus. This Tobamovirus isolates was passed through three repeated single-lesion transfers on Nicotiana tabacum cv. Xanthi-nc. This isolate caused large necrotic spots on the inoculated leaves and could not infect systemically on N. tabacum cv. Xanthi-nc, N. rustica, N. benthamiana, Datura stramonium and Tetragonia expansa. Chenopodium quinoa and N. tabacum cv. Samsoon showed necrotic local lesions on inoculated leaves and systemic necrotic local on upper leaves. Analyses of complete nucleotide sequences of the genome were comparable with other members of the genus Tobamoviruses. This isolate had very higher identity to crucifer species than to other Tobamoviruses. Especially, this isolate had very high homology (90-99% identical nucleotides) to those of Yovai mosaic virus (YoMV) and Ribgrass mosaic virus (Shanghai and Impatients isolates). The isolate is most similar to YoMV but it was seen to different biological characteristic according to host range and symptoms. These results showed that Tobamovirus isolate were collected from Rehmannia glutinosa is closely related to Ribgrass mosaic tobamovirus.

Fungicide resistance mechanisms of Fusarium fujikuroi strains against prochorlaz I. Kim (1), Y. YANG (1) (1) Chonnam National University, Gwangju, KOREA Phytopathology 101:S91

Fusarium fujikuroi is a fungal plant pathogen that causes rice Bakanae disease. A number of cases on fungicide resistance of the pathogen have been reported. Understanding the resistance mechanism is of essentials for successful disease control. This study was performed to investigate resistance mechanism of F. fujikuroi strains against prochorlaz. Fungal growth was examined in PDB with prochloraz or ATP-Binding Cassette (ABC) transporter inhibitors and PDB with prochloraz and each inhibitor. Remaining prochloraz in PDB was determined by LC-TOF/MS. Growth inhibition of the pathogens was observed in PDB containing prochloraz and each inhibitor. The concentration of prochloraz in PDB containing prochloraz and each inhibitor remained unchanged. The ratio of saturated/unsaturated fatty acids of membrane lipids of the pathogens grown with or without prochloraz is similar, suggesting membrane lipid are not responsible for the resistant mechanism. Existence of efflux pump gene was determined by amplifying gDNA with the primer designed with the conserved sequence related to ABC transporter. Sequence analyses showed 75% similarity to BcatrD gene of Botrytis cinerea isolated in Korea. Resistant strains against conazoles fungicides. These data indicate that the pathogens are capable of pumping prochloraz out of cells to decrease its toxicity. The application of prochloraz combined with ABC transporter-inhibiting chemicals would be a effective strategy to control Bakanae disease.

Genetic diversity and host range of Colletotrichum acutatum isolates obtained from several crops in South Korea J. KIM (1) (1) Chungbuk National University, Cheongiu-Si, Chungbuk, SOUTH KOREA Phytopathology 101:S91

Among 51 isolates of Colletotrichum obtained from several plants, such as pepper, Chinese matrimony vine, pear, peach, apple, avocado, and grape, 47 isolates were identified as C. acutatum by using mycological characteristics and PCR products with species-specific primers. The others were done as C. gloeosporioides. The phylogenetic relationship of all isolates of Colletotrichum spp. use the same five methods as AFLP, RAPD-PCR, and partial sequencing of the 5.8S-ITS regions and the β-tubulin 2 gene. Showing the result of each molecular method, all isolates obtained from pepper, peach and grape were belonged to A2 group, while the others from pear, apple and Chinese matrimony vine, avocado were belonged to A3 group. In the pathogenicity test with fruits of pepper, peach, and apple in vitro, 15 isolates of Colletotrichum acutatum from pepper were able to infect pear strongly, while the others from the same isolates did not infect the apple weakly. With peach, only 3 isolates among pepper isolates showed pathogenicity on peach. These results showed that there was no strong specificity of host range in C. acutatum.


In 1977, Chrysanthemum white rust, caused by P. horiana, was first detected and eradicated in PA. Until 2004, no further cases were reported. In 2004 and 2006–2010, P. horiana was detected at 82 sites (139 samples), including nurseries, greenhouses, retail stores, and residential areas, in 22 PA counties. At these sites, the PA Department of Agriculture and USDA attempted eradication plans involving quarantine, destruction, treatment, and...
Soil suppressiveness against Fusarium crown and root rot of cucumber in organic-amended soil: Occurrence and possible mechanisms

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Phytopathology 101:S92

Soil suppressiveness (SUPP) to soilborne pathogens can evolve following the incorporation of plant residues in the soil. It is characterized by reducing disease incidence and severity, despite the presence of a potent pathogen, a susceptible host and appropriate conditions for disease development. Residues of various crucifer and herb plants effectively induced SUPP to crown and root rot in cucumber plants inoculated with Fusarium oxysporum f. sp. radicis-cucumerinum (FORC) macroconidia when seedlings were planted in the tested soils 30 months after amendment. SUPP continued to be evident after repeated soil inoculations and plantings in the same soil. We studied the potential mechanisms which are involved in the evolution of SUPP after incorporation of Diplotaxis tenuifolia (wild rocket). The survival of chlamydospores of FORC decreased, by 50%, in the suppressive soil after one month of incubation. Induced systemic resistance against either FORC or Botrytis cinerea, in cucumber transplant was not evident. The composition of bacterial communities, especially Streptomycetes, in roots of plants which were grown in a suppressive soil was significantly different from those in nonsuppressive one as indicated by PCR-DGGE analysis. Quantitative PCR showed that colonization of the root tissue by FORC in soil treatments was similar at day 3 from inoculation but decreased by 60% in day 6 in the suppressive soil. Apparently, root colonization by specific microbial communities controls pathogen infection.

Interrelationships among SA, MeSA, lipids, and light in systemic acquired resistance (SAR)

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Phytopathology 101:S92

SAR is a state of heightened defense induced throughout a plant following local infection by a pathogen. Development of SAR involves synthesis of a mobile defense signal and induction of disease resistance. SA and MeSA act as a systemic acquired resistance (SAR) acquired through the vascular system to distal tissues. Results from our group argue that methyl salicylate (MeSA) serves as this mobile SAR signal in tobacco, Arabidopsis, and potato (Park et al., Science, 2007; Vlot et al., Plant Journal, 2008; Park et al., JBC, 2009; Liu et al., MPMI, 2010; Manosalva et al., MPMI, 2010). In contrast, Zeier and coworkers presented results which suggest that MeSA is not essential for SAR in Arabidopsis (Attaran et al., Plant Cell, 2009). We have identified the difference in experimental design which accounts for these conflicting results. Under certain light conditions MeSA is required for SAR signaling while under others it is not. In addition to MeSA, one or more lipid-based mobile signals have been implicated in systemic immunity by several groups. Our analyses of mutants in the lipid-transfer protein DIR1 and of plants over expressing BA/SAMT1 suggests that SAR is activated via the interplay of lipids and mobile signals. Both signals act in the same pathway to amplify MeSA responses. Our data suggests that the level of MeSA esterases in the distal tissue to facilitate conversion by MeSA esterases of the translocated MeSA to biologically active SA for induction and potentiation of defense responses (Liu et al., Plant Physiol. 2011).

Potential invasiveness of Armillaria solidipes, a tree-root-disease pathogen with a circumboreal distribution

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Phytopathology 101:S92

Armillaria solidipes (= A. ostoyae) is a root-disease pathogen that causes severe losses in growth and productivity of forest trees throughout the Northern Hemisphere. However, this species is genetically diverse with variable disease activities across different regions of the world. In North America, A. solidipes in the Colorado Plateau exists in a priori habitats and causes more disease on hardwoods in comparison with A. solidipes in the northwestern U.S.A. In China and Japan, A. solidipes causes severe root disease on Lirix spp., whereas this pathogen only rarely impacts Lirix spp. in North America. These examples indicate that A. solidipes could represent an invasive species when introduced to suitable climate scenarios. Our study demonstrates that A. solidipes across the Northern Hemisphere and the suitable climate space for each phylogenetic group can help assess potential invasive risks associated with intercontinental and interregional movement of A. solidipes.

Evaluation of wild walnut Juglans spp. for resistance to crown gall disease by propane flaming

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Phytopathology 101:S92

Crown gall (CG) disease of walnut is caused by the ubiquitous soil-borne bacterium, <i>Agrobacterium tumefaciens</i>. The most widely used rootstock Paradox, an interspecific hybrid between <i>Juglans nigra</i> and <i>Juglans regia</i>, is typically highly susceptible to <i>A. tumefaciens</i>. Identification of a durable source of resistance in wild <i>Juglans</i> species could be introgressed into commercially viable rootstocks, as an effective strategy for controlling crown gall in walnut. CG tolerant <i>Juglans</i> and <i>Pterocarya</i> spp. have been identified in a disease resistance screen conducted under greenhouse conditions. A wide range of variability in tumor formation was observed among different host genotypes. Even though CG resistance appeared to be rare in the germplasm accessions tested, <i>Juglans microcarpa</i> accessions were consistently the most resistant. Two <i>J. microcarpa</i> mother trees both generated open pollinated seedlings which exhibited increased tolerance to CG development. Rooted dormant cuttings from CG resistant selections were propagated, inoculated with <i>A. tumefaciens</i>, and continued to show CG resistance. These promising candidates are being further examined to stabilize the observed disease resistance and to be used in direct crosses with commercially viable parents as a first step towards development of crown gall resistant rootstocks.

Weed control with flaming and cultivation in corn

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Phytopathology 101:S92

Propane flaming and cultivation could be combined into a single operation as an additional tool for weed control in organic crops. Organic field experiments were conducted at the Haskell Agricultural Laboratory of the University of Nebraska, Concord, NE in 2010, and will be repeated in 2011 to determine the level of weed control and maize response to propane flaming utilized alone, or in combination with inter-row cultivation. Total of seven treatments were applied at several growth stages of maize (V3 (3-leaf) and V6 (6-leaf) with the propane doses of 20 and 45 kg/ha for the banded and broadcast flaming, respectively. Overall, weed control and maize response varied among treatments and growth stages. Cultivation at the V3 stage only, provided the poorest weed control (20%) and the lowest yield (9.7 t/ha) due to weed competition from uncontrolled weeds. The best treatment was a combination of cultivation and banded flaming conducted twice, at the V3 and V6 stages of maize. Such treatment provided about 95% weed control and yielded about 27% more than cultivation alone conducted at the same time (12.6 t/ha vs. 9.9 t/ha). All other treatments provided significantly lower weed control levels, ranging from 20–80%. Based on data from just the first year of this study, it appears that the most promising season-long weed control was achieved with a combination of flaming and cultivation treatment applied twice in field maize, at V3 and V6 stages.

Plant diseases monitoring system based on Web GIS in Jeonnam Province, Korea

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Phytopathology 101:S92
Plant diseases monitoring system, which input the disease data, analyse the disease occurrences and then inform to farmers automatically, was constructed to respond to outbreak diseases promptly. Barley, wheat and pepper diseases were scouted and analysed the disease information in real-time based on Web GIS technology in Jeonnam Province. Scab disease was not shown in middle of May, but that of wheat, naked barley, hulled barley, and malting barley showed 4.0%, 2.2%, 2.2%, and 1.2% of diseased panicles, respectively. Phythophthora blight of pepper was appeared in early of June and the severely occurred from middle of August to early of September. Anchoranose was initially occurred in middle of July but was not increase severely after that. Virus disease was first showed in early June and drastically increased from middle of July. When the disease information is shared by web GIS system, it is possible to respond effectively to outbreak diseases of plant.

Glyphosate activity on plant diseases and potential impact on plant health and yield in Roundup® cropping systems

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Phytopathology 101:S93

Laboratory and field studies in the early 2000’s suggested that glyphosate was active against rusts in glyphosate resistant wheat (P. triticina, P. striiformis) and Asian soybean rust, caused by Phakopsora pachyrhizi, in glyphosate-resistant soybeans. Further investigations into the disease control activity of glyphosate against a broader spectrum of plant pathogens demonstrated that the application of glyphosate as technical material or as Roundup® formulations can suppress the incidence and/or severity of a range of plant diseases. Experiments under growth chamber and field conditions have shown that glyphosate can suppress symptoms of disease from a range of economically important pathogens, and that applications of Roundup products have the potential to reduce yield loss in the presence of significant disease pressure. The fungicidal mode of action of glyphosate is attributed to inhibition of fungal EPSPS, with disease suppression primarily provided by systemic glyphosate. Suppression of disease may be provided from both pre and post infection applications of glyphosate. Our results indicate that glyphosate has the potential to suppress diseases caused by a wide range of fungal pathogens, and could provide incremental disease control benefits in glyphosate-resistant cropping systems.

Common ragweed (Ambrosia artemisiifolia) – a worldwide problem
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Phytopathology 101:S93

The main cause of allergy and pollen asthma in North America and Central Europe is pollen from ragweed (Ambrosia) a genus in the Asteraceae. Curren ragweed is known as Ambrosia (Ambrosia artemisiifolia L.) is rapidly spreading in Europe and has the highest weed densities in the Carpathian basin: Croatia, Hungary, and Serbia. Despite continuous efforts by the Hungarian government during the last ten years to eradicate ragweed, levels of its pollen in the air did not diminish. Ragweed infestation is heaviest in sunflower (Helianthus annuus L., the third most important crop in Hungary) fields, preceding the overgrowing majority of allergic patients in the air (in the end of the summer pollen counts reach 1000 grains m-3) even in urban areas. In the presentation we show the current situation in Europe, focusing on Hungary and discuss the most recent measures and the strategic program based on remote sensing and precision weed management methods we developed for controlling ragweed and suppressing its pollen production.

Role of rsmA in virulence of phytotoxin-producing pathohars of Pseudomonas syringae
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Phytopathology 101:S93

The gacS/gacA two-component system functions mechanistically in conjunction with the global transcriptional regulator RsmA to allow pseudomonads and other bacteria to adapt to changing environmental stimuli. Analysis of this pathway in phytotoxin-producing pathohars of Pseudomonas syringae is incomplete, particularly with regard to rsmA. Our approach was to overexpress rsmA in P. syringae strains through the introduction of pSk61, a stably maintained plasmid constitutively expressing this gene. It is convenient to study RsmA regulation using overexpression approaches as opposed to the use of knock-out mutants, as some bacteria, including pseudomonads, contain two or more rsmA alleles with redundant functions. Disease and colonization of plant leaf tissue were consistently diminished in all P. syringae strains tested (pv. phaseolicola NPS3121, pv. tabaci BR2R, and pv. syringae B728a) when containing pSk61 relative to these isolates containing the empty expression vector PM6031. Phaseolotoxin, tabtoxin, and syringomycin were also not produced in these strains when carrying pSk61. In contrast, alkaline production, biofilm formation, and the hypersensitive response were diminished in some, but not all, of these isolates under the same conditions. These results indicate that the role of rsmA varies with pathovar in the phytotoxin-producing strains of P. syringae.

Occurrence and control of Physoderma disease in China
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Phytopathology 101:S93

Physoderma disease, caused by Physoderma maydis, used to be a secondary disease on maize in China. In recent year, however, this disease becomes severer and severer with extension of new hybrids and stubble retention. The disease widely occurred of about 2 million ha and the disease incidence was about 30% to 50% in Henan, Hebei, Shandong and Anhui provinces in 2006, 2008 and 2010. Therefore, it is one of the major diseases on maize in these areas. The disease first occurred on leaves at the middle stage of maize and the disease peak was at 15–18 leaves stage. The yield loss was about 10% in general and the severe loss was from 30% to 40%. Resistance evaluations of new hybrids were conducted by artificial inoculation method. Among them, 13 high resistant hybrids were selected with the disease index lower than 11. Meanwhile, disease index of 6 susceptible hybrids were higher than 33. The result will contribute to proper usage of maize hybrids with high resistant to physoderma disease. Six kinds of fungicides including Thiophanate-methyl, Diniconazole, Triadimefon, Myclobutanil, Carbenazim and Amistar were tested in Lab condition. Results showed that all tested fungicides could restrain the germination of resting sporangia at different levels. Field trials indicated that Amistar, Diniconazole and Tebuconazole could efficient control of physoderma disease. Among them 25% Tebuconazole EC show the best control of 90% by spraying at 8–10 leaves stage.

Survival of three quarantine pathogens in a simulated aquatic system at different levels of pH
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Phytopathology 101:S93

Phytophthora ramorum, P. alni and P. kernoviae present significant threats to world biodiversity. These pathogens may spread through natural waterways and irrigation systems as exemplified by P. ramorum detection in streams and ornamental nursery effluents. However, the knowledge of their aquatic biology is scarce. Here we investigated the survival of these three quarantine pathogens in response to different levels of pH in a simulated aquatic system. Experiments were conducted using 10% Hoagland’s solution as a base medium. Treatments included a pH range from pH 3 to 11 and exposure times from a few seconds to 14 days. After overnight exposure, the highest recovery of P. ramorum, P. alni and P. kernoviae was zero, 6.9% and 12.2%, respectively. Zoospores of P. abfini survived at low rates over 14 days across all pH levels. Similar results were obtained for P. kernoviae at pH 3 to 9. Although P. ramorum zoospores failed to survive in the system, its sporangia were tolerant to all pH levels tested, and 18–43%, except for 5.9% at pH 3, survived over 14 days. Additional experiments are underway to compare the effects of pH on plant infection by zoospores and sporangia. Implications of these data on management of these quarantine pathogens are discussed.

Characterization of small RNAs derived from Tomato spotted wilt virus infection by deep sequencing
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Phytopathology 101:S93

RNA silencing is a conserved eukaryotic surveillance mechanism thought to play a role in protection against invading nucleic acids such as viruses, transposons and transgenes. Virus infection leads to accumulation of viral small RNAs (vsRNAs) at high levels. The processing of vsRNAs can be from...
viral dsRNA replicative intermediates, self complementary regions of the viral genome or from the action of RNA-dependent RNA polymerases on viral templates. The overall composition of the populations of vsRNAs generated by most plant viruses remains unknown. We have used deep sequencing techniques to characterize vsRNAs of Tomato spotted wilt virus (TSWV), a member of the genus Tospovirus, which causes economically important diseases in numerous crops in many parts of the world. The vsRNA profiles from TSWV-infected pepper, tomato, and tobacco plants were generated. Analysis of the vsRNAs indicate multiple hot spots for small RNA production from the TSWV genome, the location of these hot spots are predominantly conserved across infections of different host species. Details of the origin, distribution and abundance of TSWV vsRNAs in infected plant tissue were compiled.

Sources of resistance to Phytophthora fruit rot in watermelon plant introductions
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Phytopathology 101:S94
Phytophthora fruit rot caused by P. capsici is an emerging disease in most watermelon producing regions of Southeast U.S. Plants belonging to the core collection of U.S. watermelon plant introductions (PI) were grown in a field on raised plastic beds to evaluate for fruit rot resistance in 2009. Five fruits from each PI were harvested and inoculated with a 7-mm plug from an actively growing colony of P. capsici on V8 juice agar. The inoculated fruit were maintained in a room with high relative humidity (>95% RH) for four days. Data on length of disease lesion and intensity of sporulation were recorded for each fruit. Of the 205 PI evaluated, majority were highly susceptible. Extensive sporulation was observed on most fruit. Overall we identified 25 PI (12%) as potential sources of resistance. Twenty two (12%) of the 159 Citrullus lanatus var. lanatus PI we evaluated from the core collection, one C. colocynthis (PI 388770) and two C. lanatus var. citroides (PI 189225) showed varying levels of resistance to fruit rot. Variability in resistance reaction to fruit rot among plants of the same PI was also observed. The most resistant PI were re-evaluated in 2010. Fruit from resistant PI had significantly lower amounts of P. capsici DNA/g of fruit tissue compared to susceptible cultivars Sugar Baby and Black Diamond. Selections from the most resistant PI will be further evaluated using isolates from different states to confirm the stability of resistance.

Weeds as reservoir hosts of Tomato leaf curl virus (Begomovirus) in Tamil Nadu
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Phytopathology 101:S94
Weeds have been reported as important reservoirs for emerging Geminiviruses. The white-fly transmitted Geminivirus, especially Begomovirus are reported from regions that were earlier free of these viruses. During the last two decades they have emerged as more serious problems in a variety of crops in India. In particular, Tomato leaf curl virus (ToLCV) causes severe losses and leaf curl symptoms in tomato throughout the country. Aimed at identifying the reservoir hosts of the virus in tomato field from north eastern part of Tamil Nadu, India, we surveyed tomato plants as well as weeds growing in and around the tomato fields for the ToLCV infection for a year. ToLCV infection was confirmed by PCR using ToLCV coat protein and Rep protein specific primers that amplified a ~800 bp DNA fragment from the RNA fragrissit fruits studied. The amplicons sequenced and compared with the sequences available in NCBI confirmed their presence. Based on the above, we conclude that the weeds Euphoria hirta, and Hibiscus cannabinus L. serve as a host for ToLCV in the tomato field both during the cropping and non-cropping seasons.

Evaluation of drip applications of Revus in fungicide programs for management of Phytophthora blight (Phytophthora capsici) on bell pepper and squash
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Phytopathology 101:S94
REVUS® Fungicide (a.i. mandipropamid) was introduced in the U.S. in 2008 for control of downy mildews and diseases caused by Phytophthora spp. For all uses, including management of P. capsici on cucurbits and peppers, current labeling for Revus calls for foliar application. In view of the increasing interest in delivery of products through drip irrigation, studies were initiated to evaluate Revus applied via this route. Early trials have focused on bell peppers and cucumbers. A number of drip application regimens were evaluated including Revus solo (8 floz/A), in combination with Actigard® (0.25 or 1 oz/A), and in alternation with Presidio® (Valent) (4 floz/A). All programs started with a preplant application of Ridomil Gold® SL (1 pt/A) and included alternation with foliar applications of Ridomil Gold Copper (1 lb/A). Against intense disease pressure, all drip programs with Revus slowed development of Phytophthora blight, the most effective regimes providing ca 50 and 75% disease control on bell pepper and squash, respectively. The drip applied programs with Revus consistently performed as well or better than a foliar-only treatment and were safe on both crops. Following up on these encouraging results, we are conducting additional studies to optimize the use of Revus via drip application in programs for P. capsici on cucurbits, peppers and other fruiting vegetables.

Effects of pesticide treatments on SABP2 mediated systemic acquired resistance in plants
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Phytopathology 101:S94
Plants react to pathogen infection by inducing local and systemic acquired resistance (SAR). SABP2, a 29 kDa esterase like protein has been shown to play a critical role in the activation of SAR in plants. In agriculture, pesticides are widely used to control plant pests. Some of these pesticides have been shown to inhibit acetylcholinesterase (AChE) which converts acetylcholine to choline. Both AChE and SABP2 belong to the α/β hydrolase superfamily of enzymes and exhibit esterase activities. Inhibitors of esterase activity of AChE may also inhibit enzymatic activity of SABP2. This may prevent SABP2 mediated conversion of methyl salicylate to salicylic acid (SA) resulting in plants being unable to activate SA-signaling and hence become more susceptible to pathogen infection. In vitro studies showed that enzymatic activity of SABP2 is inhibited by organophosphates pesticides. To test, if pesticide treatment also inhibits SAR, we used 6–8 weeks old tobacco (Nicotiana tabacum cv. xanthi NN) plants. Results show that pesticide treatment compromises plants ability to mount robust SAR response in tobacco plants. Transgenic plants lacking SABP2 (due to RNAi silencing) did not show a negative effect of pesticide treatment on SAR development. Currently we are determining molecular effects of pesticide treatments on expression of defense genes. In future, emphasis while developing/choosing pesticides should be on 1) controlling pest, and 2) their effects on the pathways mediating microbial defenses of the plant.

Effect of barley chromosome addition on the susceptibility of wheat to feeding by gall-inducing leafhopper, Cicadulina bipunctata (Hemiptera: Cicadellidae)
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Phytopathology 101:S94
Cicadulina bipunctata Melchior, 1904 is distributed widely in tropical and subtropical regions of the Old World and is recognized as an important pest insect of maize. This species induces galls characterized by the growth stunting of leaves and severe swelling of leaf veins on various Poaceae such as wheat, rice and maize but not on barley. In order to clarify the mechanism of growth stunting and gall induction by C. bipunctata, we investigate the effects of barley chromosome addition on the susceptibility of wheat to feeding by C. bipunctata. As a result, degrees of gall induction and stunted growth were different among six barley chromosome disomic addition lines of wheat (2H–7H). Feeding by C. bipunctata significantly stunted the growth in 2H, 3H, 4H and 5H, but did not in 6H and 7H. Comparing to wheat, the degree of gall induction was significantly weaker in 3H and severer in 5H, respectively. Significant correlation was not detected between the degrees of growth stunting and gall induction. These results suggest that resistant genes to growth stunting exist in barley chromosomes 3 and 7, and those to gall induction are present in chromosome 3. The results also imply that growth stunting and gall induction are two independent phenomena, even though they are both induced by the feeding by C. bipunctata.

Gene expression profiling in Phytophthora phaseoli during the infection of lima bean
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Phytopathology 101:S94
Lima bean (Phaseolus lunatus L.) is an important legume crop to the state of Delaware and is susceptible to the oomycete pathogen Phytophthora phaseoli Thaxt., which causes downy mildew. In the year 2000 alone, downy mildew caused a $3 million crop loss to the industry. In this study, we have used illumina RNA-seq to identify genes in P. phaseoli orthologous to several

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effector genes in *P. infestans*, a close relative of *P. phaseoli*. To study the function of these effector proteins, we selected ten candidates with similarity to RxLRs, elicitors, NPP1 and crinklers, all of which are different classes of effectors. The above effector genes were validated by performing functional screening in Arabidopsis (*Arabidopsis thaliana*). To investigate the function of these effectors, we selected ten candidates with similarity to RxLRs, elicitors, NPP1 and crinklers, all of which are different classes of effectors. The above effector genes were validated by performing functional screening in Arabidopsis (*Arabidopsis thaliana*).

**Search for Candidatus Liberibacter spp. in citrus and orange jasmine plants and psyllids in Texas by field surveys and multi-loci PCR assays**

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Phytopathology 101:S95

Citrus huanglongbing (HLB) is associated with three species of *Candidatus Liberibacter*, ‘Ca. L. asiaticus’ (Las), ‘Ca. L. africanus’ and ‘Ca. L. americanus’. In Florida HLB was detected 5 years after the vector, the Asian citrus psyllid (ACP) was found. In Mexico and other Central America and Caribbean countries, HLB was detected 6–8 years after ACP was found. ACP was first reported in Texas in 2001, which triggered HLB surveys in citrus production and residential areas in the state. Citrus species were found adjacent to jasmine trees in dooryards. Some jasmine samples produced high Ct values above 32 in real-time PCR (qPCR). Suspect samples were recollected from these plants along with ACP adults and nymphs for DNA extraction. The primers and probe based on 16S rRNA were used to identify Liberibacter by conventional (cPCR) and TaqMan qPCR. New TaqMan primer/probe sets based on other Las genes were used to confirm the 16S rRNA results. Nested cPCR was attempted to obtain bands from high qPCR Ct value samples. Of the 16 citrus, 90 jasmine and 22 ACP samples tested, 3 jasmine trees yielded high qPCR Ct values with 16S rRNA and some of the new primer/probe combinations from Las genes, but tested negative with cPCR. The combination of qPCR primer sets may help resolve the high Ct value samples during subsequent testing.

**First report of Sweet orange scab in U.S.A.**

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Phytopathology 101:S95

Sweet orange scab caused by *Elsinoe australis* was first identified in Brazil in 1937, and has also been reported from Kenya in 2001 (natsudaidai pathotype). It is reported to mainly affect the fruit of sweet oranges and mandarins, and until the detection of *Mycosphaerella* by molecular (1), assays, was distinguished from *Elsinoe fawcettii*, the causal fungus of sweet orange scab, by host range studies and the rarity of foliar symptoms. In 2010, a lemon fruit with scab symptoms was collected in Spring TX (near Houston) and tested negative with cPCR and had no typical symptoms and will be monitored. The combination of qPCR primer sets may help resolve the high Ct value samples during subsequent testing.

**Antimicrobial lipopeptide iturin induce systemic resistance of Arabidopsis thaliana**

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Phytopathology 101:S95

Iturin is an antimicrobial lipopeptide produced by antagonistic strains of *Bacillus spp.*, and is deduced to play key roles in biological control for several kinds of plant diseases; however, the function of iturin in disease suppression is still unclear. Here, we report that a novel function of iturin as an elicitor for *Arabidopsis thaliana*. The root-treatment of purified iturin to hydroponic *Arabidopsis thaliana* plants results in symptoms that are characteristic of Colletotrichum disease on leaves. Gene expression analyses of host plant revealed that the treatment of iturin to root induced some systemic resistant related genes.

**Evaluation of Mycosphaerella polygoni-cuspidati for classical biological control of Japanese knotweed (Fallopia japonica)**

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Phytopathology 101:S95

*Mycosphaerella polygoni-cuspidati* was selected as a potential classical biological control (CBC) agent for *Fallopia japonica* according to the results of the surveys and screening. The *M. polygoni-cuspidati* was re-described and neotypified based on not only morphology but also the internal transcribed spacer ITS region including the 5.8S ribosomal DNA (rDNA) sequences. Field observations revealed that this pathogen had a reduced life cycle, with only spermogonia and pseudothecia being formed. Under controlled environmental conditions, disease development of *M. polygoni-cuspidati* mycelia on *F. japonica* was assessed under several factors including leaf age, dew-period duration and post-dew temperature. *F. japonica* leaves at younger leaf stages of 7–9 days after opening were more shown to be caused by *Impatiens necrotic spot virus* (INSV). Sequence analysis of N and NSs genes confirmed that lettuce-infecting INSV isolates were similar to previously characterized INSV isolates, and RT-PCR was used to investigate the INSV inoculum source(s). INSV was detected in *Frankliniella occidentalis* from infected and non-infected lettuce and some asymptomatic weeds, such as malva and shepherd’s purse. This indicates that weeds are an inoculum source. To investigate properties of INSV proteins, the NSs and NSm were expressed alone or as fusions with GFP for subcellular localization and silencing suppressor experiments. Both NSs and NSm were expressed in *Nicotiana benthamiana* plants via *Agrobacterium tumefaciens*-mediated transient expression. Confocal laser scanning microscopy results showed that NSm formed punctate bodies in the cell membrane or wall, whereas NSs localized at the nuclear periphery and cytoplasm. Silencing suppressor experiments in *N. benthamiana* 16G F gene transgenic plants, and RNA binding assays with NSs and NSm are ongoing. Based on our preliminary data and previous studies on other tospoviruses, NSs may be the silencing suppressor of INSV.

**The detection of Ceratocystis fagacearum in Texas live oak using real-time polymerase chain reaction**

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Phytopathology 101:S95

The diagnosis of oak wilt depends on isolation of the pathogen, *Ceratocystis fagacearum*, from wood tissues of symptomatic trees plated onto a growth medium such as acidified potato dextrose agar. This technique is time consuming, inefficient and has many limitations, including a long incubation period which often produces the negative results. With the objective of developing a specific molecular detection system, 16 GenBank accessions of *Ceratocystis* ITS DNA sequences were used to generate a BLAST alignment to look for regions of variation that could be used to discriminate for *C. fagacearum* in a quantitative real-time PCR assay. Two regions, including 341 to 510 bp (ITS1) and 651 to 820 bp (ITS 2) were selected and further compared with 81 data bases of fungal DNA sequences. Primers and fluorescent labeled probes specific for *C. fagacearum* were designed using Primer Express® software (Applied Biosystems). These primer/probe sets successfully detected the pathogen from cultured spor suspensions and purified, target DNA without amplifying closely related, non-target fungal species. The ITS primer/probe set CIP2 consistently detected *C. fagacearum* from different symptomatic tissues that were confirmed by pathogen isolation. The preliminary testing of this technique demonstrates the potential for this tool to be a significant breakthrough in the diagnosis of oak wilt and invaluable in the study of this destructive tree pathogen.

**Functional analysis of NSs and NSm genes of Impatiens necrotic spot virus found in Salinas valley, California**

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Phytopathology 101:S95

In the 2006-2007, lettuce plants in Monterey County California showed necrotic spotting and mottle/mosaic symptoms, which were subsequently shown to be caused by *Impatiens necrotic spot virus* (INSV). Sequence analysis of N and NSs genes confirmed that lettuce-infecting INSV isolates were similar to previously characterized INSV isolates, and RT-PCR was used to investigate the INSV inoculum source(s). INSV was detected in thrips (*Frankliniella occidentalis*) from infected and non-infected lettuce and some asymptomatic weeds, such as malva and shepherd’s purse. This indicates that weeds are an inoculum source. To investigate properties of INSV proteins, the NSs and NSm were expressed alone or as fusions with GFP for subcellular localization and silencing suppressor experiments. Both NSs and NSm were expressed in *Nicotiana benthamiana* plants via *Agrobacterium tumefaciens*-mediated transient expression. Confocal laser scanning microscopy results showed that NSm formed punctate bodies in the cell membrane or wall, whereas NSs localized at the nuclear periphery and cytoplasm. Silencing suppressor experiments in *N. benthamiana* 16G F gene transgenic plants, and RNA binding assays with NSs and NSm are ongoing. Based on our preliminary data and previous studies on other tospoviruses, NSs may be the silencing suppressor of INSV.
susceptible than the older leaves, especially those at the older than 13 days after opening. When adequate dew was provided, severe defoliation was observed over the dew-period temperature range of 15 to 20°C. With a dew period of at least 18 h, leaf defoliation was occurred, but the disease incidence was greater on plants submitted to 42–48 h dew periods. The optimal post-dew temperature for disease development was 19–21°C. Preliminary host specificity tests using UK & plant species showed that M. polygoni-cuspidati is highly specific to F. japonica. These results indicate that M. polygoni-cuspidati has high potential as a CBC agent for an invasive weed, F. japonica.

Is there any other elixir of life on this planet?
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Phytopathology 101:S96

Excessive and indiscriminate use of fertilizers and pesticides had caused several ecological problems. Hence, locally available natural farm products like Annamalai mixture (cow urine, cow dung, sheep dung, poultry litter and neem cake), turmeric powder, hydrated lime were tested against various plant pathogens and vectors either alone or in combination. Spray of farm natural products particularly Annamalai mixture at biweekly intervals for 15 times have resulted in manifold increase (3–7 times) in the yield of several crop plants (cereals, pulses/legumes, oil seeds, cotton and vegetables). They also significantly reduced the major diseases of rice and pulses/legumes; bacterial blight of cotton, red rot of sugarcane, anthracnose of chilies, damping – off and killed or repelled aphids, white flies, leaf hoppers and mealy bugs completely. Livestock excreta treated rhizosphere soil exhibited maximum number of bacteria, followed by actinomycetes and fungi. For fungal and bacterial excreta treated test crops, bacterial disease treated urine whereas for viral diseases died extract worked well. Among all the animal excreta tested cow, sheep and poultry were found to be the best sources. The volatile ammonia, silica in livestock excrements, curcumin (turmeric powder), calcium (hydrated lime) and azadiractin (neem cake) were responsible for the toxic effect and the enhanced yield might be due to various macro and micro elements present in them. Hence, Annamalai mixture is yet another elixir of life in addition to water on this planet, earth.

Management of leaf curl diseases by eco-friendly methods
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Phytopathology 101:S96

Tomato and chillies are the important crops grown in many countries. The leaf curl caused by leaf curl virus transmitted by whiteflies (Bemisia tabaci) is the most important one. Hence, locally available natural products were tested against these diseases and the insect vector. Leaf extract of Prospis chilensis 75%, Azadiracta indica 100%, cow dung 50%, sheep dung 75% and cow urine 100% recorded total mortality of whiteflies. Cow dung, cow dung plus sheep dung combination spray as well as leaf dip method (50%, 1:1/v) recorded total prevention of whiteflies settlement. When mixed with the viral inoculum, the combination treatment caused no disease in both the systemic as well as indicator plants. No virus was recovered on back inoculation. A lack of information on pathogenicity of these microorganisms prompted us to determine whether they possess a cellulase gene, an essential prerequisite for virulence in Xanthomonas. Two sets of degenerates primers produce overlapping, about 600-bp amplicons that, when searched by BlastX, showed about 92% amino acid similarity to the protein product predicted for the EngA gene of X. albilineans. The results can be interpreted as indicative of the potential for pathogenicity on other hosts perhaps.

Salmonella enterica moderates Pectobacterium carotovorum populations and virulence on lettuce
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Phytopathology 101:S96

Contamination by plant or human pathogens renders fresh produce unmarketable. Infection by the phytopathogen Pectobacterium carotovorum causes water-soaked lesions and rapid maceration of plant tissues. Salmonella enterica serovar Saintpaul caused multisite salmonellosis outbreaks linked to peppers in 2008 and alfalfa in 2009. This enteric human pathogen survives in the environment between warmed-blooded hosts and plants have become an important vehicle to humans. Phytopathogens improve the survival of human pathogens on plants but the converse interaction is unknown. We examined the interaction of S. enterica Saintpaul and P. carotovorum on lettuce by measuring population, pH, and virulence (lesion length). Detached lettuce leaves inoculated at the midrib with 10^6 CFU of S. enterica Saintpaul, P. carotovorum WPP359, or a P. carotovorum ΔbudB mutant were sampled every 24 h, up to 96 h. Alternatively, leaves were co-inoculated with 10^5 CFU each of S. enterica and WPP359 or ΔbudB. At 72 h and 96 h, S. enterica populations were higher in the presence of P. carotovorum while populations of both P. carotovorum strains declined in the co-inoculations compared to lone inoculations. Lesion lengths were substantially larger, and pH significantly higher, in the WPP359 treatment compared to ΔbudB, S. enterica co-inoculations, and controls. These results indicate a potential role for human pathogens in plant disease development with implications for plant health and food safety.

Strategies of biological and symbiotic control of citrus variegated chlorosis by endophytic bacteria
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Phytopathology 101:S96

Citrus Variegated Chlorosis (CVC) is an economically important and destructive disease caused by Xylella fastidiosa (Xf) and transmitted by sharpshooter insects. One factor that may confer resistance to CVC is the endophytic microbial community colonizing individual C. sinensis plants. Our results suggest that there are interactions between Xf and endophytic bacteria present in the xylem of sweet orange, and that these interactions, particularly those involving Curtobacterium flaccumfaciens (Cf), may affect disease progress. Symbiotic control is a new strategy that uses symbiotic endophytes as biological control agents to antagonize or displace pathogens. Also, candidate endophytes for use in symbiotic control of CVC must occupy the xylem of host plants and attach to the precibarium of sharpshooter insects in order to have access to the phytopathogen. We demonstrated the transmission, colonization, and genetic stability of several candidate endophytes in the precibarium of sharpshooter insects in order to have access to the phytopathogen. We propose the endophytic bacterium as a biocontrol agent. We propose the endophytic bacterium as a biocontrol agent. We propose the endophytic bacterium as a biocontrol agent. We propose the endophytic bacterium as a biocontrol agent.
receive notification when a taxonomic group is reported in the geographic areas they are monitoring, and allow Cooperative Invasive Species Management Areas (CISMAS) to keep records on treatments to stop the spread of invasive species. This system is part of the Center for Invasive Species and Ecosystem Health at the University of Georgia. Several new projects are now underway to expand the scope of the system, the completeness of the data, and the functionality to the end user. Any groups interested in being a part of these new efforts can go to www.EDDMapS.org to learn more.

Tomato powdery mildew may be significantly reduced by choice and management of irrigation system in the Brazilian Middle West

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Phytopathology 101:S97

Powdery mildew (PM, Leveillula taurica) is a limiting factor for the irrigated tomato (Solanum lycopersicon) winter crop in the Brazilian Midwest. The winter season is dry with a large thermal range and frequent dew formation. A detailed study of the relation between irrigation methods and Leveillula mildews has not been attempted. We studied the effect of irrigation methods on the severity of tomato PM for two years (2009 and 2010), in a RCBD with 3 replicates and 100 plants per plot. The following systems were studied: Drip with one line (D1L); or two lines (D2L); Drip with one line and plastic mulch (DPM); or corn mulch (DCM); Furrow (FUR); low pressure microsprinkler with one line (MIC); and conventional overhead (COV). Irrigations were performed at each one of two soil moisture tensions: 15-30 kPa (high moisture) or 30-60 kPa (moderate moisture). FUR and DCM were irrigated only at the moderate level, totalling 12 treatment-combinations. PM was evaluated weekly and the Gompertz model was adjusted to severity data. All moderately irrigated drip, tape and furrow treatments, as well as high moisture drip treatments, had high Ymax values (>87%), disease progress rates (t between 0.052 and 0.065) and AUDPCs. Microsprinkler irrigation at high moisture caused a delay in disease progress and intermediate severity levels. Lowest Ymax (13%), t (0.013 to 0.018) and AUDPCs were observed in both overhead irrigated treatments. PM severity varied among years, but results were consistent for both years.

Transgenic plants expressing antimicrobial lactoferrin protein are resistant to Rhizoctonia solani

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Phytopathology 101:S97

Lactoferrin (LF), a cationic iron-binding glycoprotein of the transferring family is present in milk, tears, saliva, and mucous secretions of most mammals. LF is known to exert a broad-spectrum primary defense activity against pathogens and fungi. The Bovine lactoferrin (BLF) gene was introduced to tobacco (Nicotiana tabacum var Xanthii) and Arabidopsis (Arabidopsis thaliana) plants with Agrobacterium tumefaciens strain C58C1 containing a plasmid construction carrying a modified BLF cDNA. Plants expressing BLF were evaluated for resistance against a delay in disease progress and intermediate severity levels. Infection of BLF cDNA into susceptible tobacco and Arabidopsis lines was confirmed by Southern blot and the expression of full-length LF transcript and protein was also detected by Northern and Western blots, respectively. Transgenic lines segregating for a single locus insertion were identified and used for disease resistance assays. Detected leaves of transgenic tobacco plants exhibited high levels of Rhizoctonia resistance. In addition, transgenic Arabidopsis seedlings were resistant to R. solani and prevented damping off symptoms. Use of BLF gene is a potential new approach to consider for control of diseases caused by fungal pathogens.

Epistatic involvement of plasmodesmal localized protein and malic acid transporter in aerial pathogenesis and belowground rhizobacterial recruitment

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Phytopathology 101:S97

Beneficial soil bacteria confer immunity against a wide range of foliar diseases by activating plant defenses. Our recent work demonstrated that foliar infection by Pseudomonas syringae pv tomato (PstDC3000) induces recruitment of Bacillus subtilis (FB17), a beneficial rhizobacteria. We also show the induction of a malic acid (MA) transporter (ALMT1) post PstDC3000 infection, leading to increased MA titers in the rhizosphere. Intra-plant signaling under distress conditions, especially between aboveground and belowground tissues is potentially complex due to the involvement of significant physical distances. Although, cell to cell signaling through plasmodesmata has been demonstrated in various physiological and developmental plant responses, but how plasmodesmata mediates cell to cell signaling to infiltrate innate immunity is not well understood. Recently, a plasmodesmata localized protein (PDLPS) is shown to be transiently expressed during a foliar PstDC3000 infection. We therefore hypothesize that post PstDC3000 aerial infection; plants may relay a shoot-to-root signal involving a member of the aluminum-activated ALMT1 to recruit FB17 in the rhizosphere. Concomitantly, the PDLPS over-expression (35S::PDLPS) showed higher levels of ALMT1 compared to PDLPS knock-out (pdlp5). The 35S::PDLPS secreted higher titers of MA compared to the almt1 and pdlp5. In addition, the mutants almt1 and pdlp5 exhibit reduced biofilm formation by FB17. Furthermore, both pdlp5 and almt1 were susceptible to PstDC3000.

Management of peach blossom blight canker development with biorational fungicides

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Phytopathology 101:S97

Infection of peach flowers by Monilinia spp. can result in formation of cankers that are important sources of inoculum for subsequent development of brown rot on fruit. Results of a preliminary study in 2008 indicated that the biorational fungicides Bacillus subtilis (Serenade MAX), potassium bicarbonate (Kaligreen), and neem oil extract (Trilogy) provided good to excellent control of canker formation. To further examine efficacy, low and high rates of these fungicides, along with a cyprodinil (Vangard) standard, were applied to trees in an “Encore” orchard in 2009 and 2010 using a RCBD with four blocks. Treatment applications were made at 5-10% bloom, 75-100% bloom, and 75-100% petal fall stages. Disease was assessed by counting blossom blight cankers that formed by mid-summer on 20 flowering shoots per tree. Disease incidence was expressed as percent shoots with canker and canker density as number of cankers per shoot. Analyses of combined 2009–2010 data revealed that all treatments significantly reduced canker incidence and density. Non-treated trees had an average 16.9% incidence, while treated shoots ranged from 0 to 9.4% incidence. Furthermore, disease levels for five of the six biorational treatments, which provided 59 to 85% control, were not significantly different from the standard. These results suggest that some biorational fungicides may be directly substituted for conventional fungicides during bloom without significant loss in blossom blight control.

Characterization and epidemiological aspects of a novel badnavirus infecting fig

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Phytopathology 101:S97

Fig mosaic (FM) is the most common virus disease of fig (Ficus carica) with symptoms varying greatly between locations and cultivars, probably because of the number of viruses infecting the crop. A novel badnavirus was discovered in FM trees in Arkansas. The complete genome was obtained using PCR-based rolling circle amplification and phylogenetic analysis revealed its novel association to Citrus mosaic virus and the fig swollen shoot virus. A survey of FM material from the National Germplasm Repository in Davis, CA, and Fayetteville, AR, was conducted and the new virus was widespread in both locations with over 75% FM trees (30/40 samples) tested positive but was also found in asymptomatic material. Virus diversity was investigated using 22 isolates all of which were very similar with nucleotide identities ranging from 99–100%. Infected tissue was mechanically inoculated onto 19 indicator species and the badnavirus can infect pumpkin, soybean, English pea and several tobacco species. The new virus may be of concern to the fig growing areas due to the apparent ease of mechanical transmission, which may in turn account for its widespread presence in fig trees.

Reducing damage to root-knot nematode with fluensulfone (formerly thiazosulfene) in cucumbers and peppers

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Phytopathology 101:S97

Root-knot nematode (Meloidogyne incognita) is the most prevalent and damaging nematode affecting vegetable crops in the southeastern U.S. Fumigants such as methyl bromide (MB) and 1,3-dichloropropene have been used extensively to control this nematode, but they are expensive, require specialized application equipment, are hazardous to handle, and are under heavy regulation. An efficacious, non-fumigant nematocide such as fluensulfone would negate many of these problems. In the fall of 2010,
fluensulfone was compared to several fungimants and oxamyl on slicing cucumber and bell pepper. Combinations of fungimant and non-fungimant nematicides were evaluated as well. Fluensulfone and oxamyl were soil-incorporated and 1,3-dichloropropane was applied with a yetter rig prior to laying plastic. All other fungimant treatments were applied using soil-injection/plastic laying equipment and all treatments were tarped with VIP plastic mulch. Fluensulfone and oxamyl were subsequently applied several weeks after planting through drip irrigation. Fluensulfone alone and in combination with oxamyl significantly reduced nematode galling compared to oxamyl alone and non-treated plots in both cucumber and pepper and was similar to fungimant-treated plots. No differences in yield were noted in the cucumber trial. Pepper yields were reduced by MB, chloropiramine and metam sodium treatments due to phytotoxicity.

Components of resistance to Phytophthora nicotianae in doubled-haploid lines of tobacco possessing a novel source of resistance

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Black shank of tobacco, caused by the oomycete Phytophthora nicotianae, is an important disease of tobacco primarily managed by the deployment of partial and complete resistance genes. Following the widespread occurrence several race 1, new sources of resistance are needed. Beinhart 1000 (BH 1000) is highly resistant to all races of P. nicotianae. Doubled-haploid lines from a cross of BH 1000 and the susceptible variety Hicks were evaluated for black shank resistance and a linkage map with 24 linkage groups was created. QTLs on linkage groups (LG) 4 and 9 accounted for 43% of the phenotypic variation for expanded survival; the QTL on LG 4 is a novel source of resistance. Forty three doubled-haploid lines with genomic regions from BH 1000 or Hicks on LG 4 and/or LG 8 from were selected and evaluated in greenhouse tests along with both parents for incubation period, percent root rot, and secondary inoculum production. Genotypes with LGs 4 and 8 from BH 1000 had increased incubation periods and decreased root rot compared to genotypes with LGs 4 and 8 from Hicks. The effects of the two LGs were additive and genotypes with both QTLs were significantly different from genotypes with only one QTL for all measured components of resistance. The previously unidentified QTL on LG 4 may provide growers with a new source of resistance to the black shank disease.

Implication of antibiotic in the biocontrol of Clavibacter michiganensis causing bacterial wilt and canker of tomato by Pseudomonas spp.

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Phytopathology 101:S98

2,4-diacyetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) and hydrogen cyanide (HCN) are antimicrobial metabolites produced by Pseudomonas spp. which show high biological activity against fungal plant pathogens. However, we know little about the impact of these metabolites on bacterial plant pathogens and the diseases they cause. In this study, two antimicrobial metabolite-producing Pseudomonas spp. strains (LBM223 producing PCA and LBM300 producing DAPG and HCN) as well as their respective mutants (LBM223 PCA-, LBM300 DAPG-, and LBM300 HCN-) were used to study their in vitro and in planta growth effects on Clavibacter michiganensis subsp. michiganensis (Cmm) growth using in vitro confrontational assays and in planta experiments performed under soil conditions. Under in vitro conditions, both LBM223 and LBM300 significantly inhibited the growth of Cmm. Larger inhibition zones were observed with LBM223 and LBM300 when compared to their respective mutants, indicating that PCA, DAPG and HCN all contributed to Cmm growth inhibition. In plant assays, inoculation with all isolates significantly reduced disease symptoms and Cmm population in the rhizosphere, while inoculation with LBM223 did not yield any effect. Interestingly, inoculation with LBM300 DAPG- or HCN- also did not yield any effect, suggesting that the production of both DAPG and HCN is required under rhizosphere soil conditions for the biocontrol of Cmm.

Effect glucorafano isolated of broccoli florets on the germination of Colletotrichum gloeosporioides spores

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Anthrax caused by Colletotrichum gloeosporioides, is the most important postharvest disease in mango producing areas worldwide, the strategy most used to control disease, is the pre-and post-harvest treatment with fungicides, but their use is increasingly restricted due to public awareness of hazardous waste in the fruits. Glucosinolates are natural products containing nitrogen and sulfur and its antimicrobial activity has been shown in other research. For this work, we collected fruits of mango, with symptoms of anthracnose: from them it was isolated and identified the fungus Colletotrichum gloeosporioides. The spores of the pathogen were placed on PDA with different glucorafan concentration (1.54, 0.92, 0.46, 0.15, 0.02 y 0 mug mL–1) isolated from broccoli florets. We measured spore germination until the control treatment show the highest percentage of germination. The median lethal concentration was 0.65 mug mL–1 and the concentration that completely inhibited the germination of spores was 0.97 mug mL–1.

Use of disease-suppressive Brassica rotation crops in potato production: Overview of 10 years of field trials

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Phytopathology 101:S98

Disease-suppressive Brassica rotation crops have shown promise for management of soilborne diseases and enhanced yield in a variety of crop production systems. Over the last 10 years, numerous field trials have focused on how to best use Brassica crops in potato rotations in the Northeast, including which crops to use, what diseases are affected, and how to implement and manage these crops (as cover, harvested, or green manure crops). A summary of over 70 individual trials indicated that, although results varied by field and year, positive effects have been observed in most trials. Yield was significantly improved in 52% of the trials, with increases up to 38%. Black scurf was significantly reduced in 70% of the trials, with reductions up to 95% and an average reduction of 30% relative to typical rotation crops. Common scab was reduced in 40% of the trials, with reductions up to 50%. Powdery scab and Verticillium wilt were also reduced in most of the trials where they occurred. Overall, mustard green manures worked best for reducing most soilborne diseases, but rapeseed green manure was best for black scurf. In general, green manures provided the best results, but crops harvested for seed also significantly reduced disease. However, due to the short growing season, Brassica crops were not effective as a fall cover crop. This research demonstrated that Brassica rotation crops can substantially reduce soilborne disease problems, but cannot completely control them.

Basis for inhibition of Pyrenophora teres by Laetisaria arvalis, a scanning and transmission electron microscopic study

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Phytopathology 101:S98

The broadly occurring foliar disease of barley, net blotch is caused by Pyrenophora teres, an ascomycete and could result in significant yield loss in cereal production. Pyranophora teres was shown to produce the compound glucorafano, which has been reported to have biological control activity over some plant pathogens. In a preliminary experiment, L. arvalis inhibited growth of P. teres on agar plates. The observation however, did not elucidate the mechanisms of the P. teres inhibition by L. arvalis. This research was initiated to utilize electron microscopy techniques to examine the interaction between L. arvalis and P. teres to study the basis for previously observed inhibition. Scanning and transmission electron microscopy were used to examine the interaction of the two fungi. To date, our microscopy data indicated structural changes of the hyphae in both P. teres and L. arvalis as the fungi interact. Additional examination of interacting colonies of the two fungi growing on agar shows loss of structural integrity in P. teres hyphae, whereas the L. arvalis hyphae remain intact. This includes the formation of large perforations in P. teres hyphae resulting in its growth inhibition. This observation strongly confirms the likely inhibition of P. teres by L. arvalis. Additional experiments are in progress to better understand this interaction at the subcellular level to serve as basis for the development a biological control system of P. teres with L. arvalis.

Detection of Pyrenophora teres in cornida and barley seed by PCR, a technique for rapid diagnosis of infestation

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Phytopathology 101:S98

Pyrenophora teres, the causal agent of net blotch of barley survives as seedborne mycellum or pseudothecia in infested host residues. Besides the common netlike and occasional spot form symptoms on barley leaves, diffused dark or pale symptoms which are difficult to distinguish from other
fungi are produced on kernels. Examination of conidia is considered the most accurate way to diagnose net blotch of barley. We developed a PCR technique to detect *P. teres* in conidia and seeds. In this technique, conidia and barley seeds showing symptoms were first homogenized in Extract-N-Amp Plant PCR Kit (Sigma-Aldrich) extraction solution and diluted with another solution from the kit to sidestep standard DNA extraction. Freeze dried mycelial cultures of *P. teres* and *P. teres* *Maculata* were treated as seeds and conidia to serve as controls. Aliquots of the homogenate were added to PCR reaction and subjected to amplification using *P. teres* actin based PTACTIN980 and ITS primers. Sizes of amplicons from infested seeds and conidia which were resolved on agarose gel correlated with amplicons from the control *P. teres* cultures. The amplicons, purified from gels, sequenced and compared by alignment confirmed the detection of *P. teres*. The technique will accelerate positive detection of *P. teres* in infested barley seeds from diseased kernels by positively distinguishing it from other fungi and hasten the diagnosis from conidia.

**Epiphytic populations and the effect of UV light on Cladosporium spp. found on blueberries**

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Phytopathology 101:S99

Cladosporium rot (*Cladosporium spp.*) is a common disease affecting blueberries (*Vaccinium corymbosum*) and other crops in Chile. In this study, the epiphytic populations on blueberries and the effect of UV light on *Cladosporium spp.* were studied. Isolations were made from samples of flowers or immature fruits that were agitated for 5 min in 1:3 or 1:6 w/v 0.05% Tween, respectively. The resulting suspension (0.05 ml) was plated on PDA plus antibiotics and Igepal for 14 days at 20°C to determine the fungal colonies that were present. The effect of UV-A (λ = 361 nm) and UV-C (λ = 254 nm) on the inactivation of the conidia of *C. cladosporioides* and *C. herbarum* was investigated. Conidial suspensions (10^6 conidia/ml) were first subjected to UV-A (0.0, 0.05, 0.1 or 0.15 J/cm^2) or UV-C (0.0, 0.05, 0.1 or 0.15 J/cm^2), and then 0.1 ml of the suspension was immediately plated on PDA plus Igepal for 48 h at 20°C. The total number of colonies was counted. Species of *Cladosporium*, *Botrytis*, *Penicillium*, *Alternaria* and yeasts were isolated from the flowers and berries. *Cladosporium* spp. and yeasts were the most abundant species. The conidia of *Cladosporium* spp. exhibited a high resistance to UV-A and UV-C, which drastically differed from the high UV sensitivity of *B. cinerea* that was found in this study. The melanin pigments in conidia and seeds. In this technique, conidia and barley seeds showing symptoms were first homogenized in Extract-N-Amp Plant PCR Kit (Sigma-Aldrich) extraction solution and diluted with another solution from the kit to sidestep standard DNA extraction. Freeze dried mycelial cultures of *P. teres* and *P. teres* *Maculata* were treated as seeds and conidia to serve as controls. Aliquots of the homogenate were added to PCR reaction and subjected to amplification using *P. teres* actin based PTACTIN980 and ITS primers. Sizes of amplicons from infested seeds and conidia which were resolved on agarose gel correlated with amplicons from the control *P. teres* cultures. The amplicons, purified from gels, sequenced and compared by alignment confirmed the detection of *P. teres*. The technique will accelerate positive detection of *P. teres* in infested barley seeds from diseased kernels by positively distinguishing it from other fungi and hasten the diagnosis from conidia.

**Diaporthe/Phytophthora complex associated with stem cankers of blueberry in Chile**

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Phytopathology 101:S99

Commercial blueberry (*Vaccinium corymbosum*) plantings extend across a southern region of the U.S. where the reniform nematode, *Rotylenchulus reniformis* and the root knot nematode *Meloidogyne incognita* predominate. Soybean differential having resistance to *Heterodera glycines* were used to evaluate potential resistance to *Rotylenchulus reniformis* and *Meloidogyne incognita*. Four trials were established with *Pis 437654, 209332, 90763, 89772, 548316, 548658, 88788, 97100, 548402, Hutcheson and Williams 82.* Soybeans were inoculated with *M. incognita* or *R. reniformis* and grown for 60 days. The *R. reniformis* population, when compared to the standard PI548658, was lower on all the differentials except for PI97100. The PI97100 genotype supported a 10% higher population of *R. reniformis* than PI548658. Five of the differentials supported a 10% greater population of *M. incognita* than PI548658. The *Pis* 437654, 209332, 89772, 548416, and 97100 all supported higher levels of *M. incognita* than PI 548658. The *Pis* 90763, 88788, and 548402 supported 85 to 50% fewer root-knot. Hutcheson, supported both *R. reniformis* and *M. incognita*, while Williams 82 increased only *R. reniformis* populations. Results indicate that the genes for resistance to *H. glycines* present in some *Pis* parentage may also influence susceptibility and resistance to *R. reniformis* and *M. incognita*.

**Fungicide resistance in Czech cucurbit powdery mildew populations**

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Phytopathology 101:S99

A total of 113 cucurbist powdery mildew (CPM) isolates (67 *Golovinomyces cichoracearum* (Gc), 46 *Podosphaera xanthii* (Px)) collected in the Czech Republic (2005–2008) were screened for fungicide resistance (fenarimol, dinocap and thiophanate-methyl; benomyl - inefficient control). Sixty CPM isolates (36 Gc, 24 Px) from 2007–2008 were also tested for sensitivity to azoxystrobin. Sensitivity was determined by a modified leaf-disc bioassay with five concentrations. Significant differences among fungicides and years were observed. Resistant and/or tolerant isolates of both CPMs were found in different locations. Isolates collected in 2005 exhibited lower sensitivity to fenarimol (Rubigan 12 EC) and dinocap (Karathane LC) compared to previous years; however in 2006–2008 a high level of sensitivity to these fungicides was detected, all isolates of both CPMs were controlled by the recommended concentration (36 µg/ml fenarimol, 105 µg/ml dinocap). Benomyl (Fundazol 50 WP) and thiophanate-methyl (Topsin M 70 WP) would be inefficient for CPM, most isolates screened were highly resistant, with limited or profuse sporulation at the recommended as well as higher concentrations. Sensitivity to azoxystrobin (Ortiva) decreased from 2007 to 2008 when 45% of CPM isolates (Gc and Px) were tolerant or resistant to the recommended concentration (500 µg/ml), most also tolerated 1000 and 2000 µg/ml. This research was supported: QH 71229, MSM 6198959215, IGA PrF_2011.

**PemK toxin encoded by the *Xylella fastidiosa* IncP-1 plasmid pXF-RIV11 is a ribonuclease**

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Phytopathology 101:S99

Stable inheritance of the IncP-1 plasmid pXF-RIV11 in *Xylella fastidiosa* is conferred by the pemI/pemK plasmid addiction system. PemK serves as a toxin inhibiting bacterial growth; PemI is the corresponding antitoxin that blocks activity of PemK toxin by direct binding. PemK toxin and PemI antitoxin were over-expressed in *Escherichia coli* and activities of each were assessed. Purified PemK toxin specifically degraded single-stranded RNA but not double-stranded RNA, double-stranded DNA, or single-stranded DNA. Addition of PemI antitoxin blocked nuclease activity of PemK toxin. Purified complexes of PemI bound to PemK exhibited minimal nuclease activity; removal of PemI antitoxin from the complex restored nuclease activity of PemK toxin. Sequencing of 5′ RACE products of RNAs digested with PemK revealed a preference for cleavage between U and A residues of the trinucleotide UAC. Nine single amino acid substitution mutants of PemK toxin were constructed and evaluated for growth inhibition, nuclease activity, and PemI binding. Three PemK point substitution mutants (R3A, G16E, and D79V) that lacked nuclease activity did not inhibit growth. All nine PemK mutants retained the ability to bind PemI antitoxin. Collectively, the results indicate that mechanism of stable inheritance conferred by the pXF-RIV11 pemI/pemK plasmid addiction system is similar to that of the prototype pemI/pemK addiction system of *E. coli* plasmid pR100.

**Fungicide screening and application for the control of walnut anthracnose caused by *Glomerella cingulata***

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Phytopathology 101:S99
Anthracnose is one of the most important disease occurred in leaves, petioles, and even in nuts of Walnut tree. Infected leaves and twigs are malformed, the leaves deformed during growing season. Severely infected nuts became black and subsequently dropped. The disease occurrence cause the dramatic decrease in yield and quality of nut production. The disease was first reported in Korea at 1987, and the damage rate is annually increasing about 15~30% in Youngdong, Gimcheon, Buyeo, Gongju, and Eumseong area, where walnut tree is densely planted, and not managed well. In order to find and apply effective fungicides for the control of the disease, 8 fungicides including azoxystrobin (20%) were screened in vitro at the diluted concentration of from 500 to 4,000 times. Tebuconazole (25%) and Fluziamin (50%) showed the highest inhibition in mycelial growth of the pathogenic fungus. Application of tebuconazole (emulsifiable concentrate) at the diluted concentration of 2,000 times on the infected leaves showed high control value of 92.2%, while treatment of fluziamazin (wettable powder) showed satisfactory control value of 82.5% on the infected nuts.

Identification of quantitative trait loci conferring partial resistance to Phytophthora sojae in soybean PI 427106

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Phytopathology 101:S100

Phytophthora root and stem rot caused by Phytophthora sojae is a destructive disease that limits soybean yield around the world. Fifteen resistance genes (Rps) to P. sojae have been identified, but adaptation by the pathogen has made these genes ineffective. In addition to Rps genes, partial resistance controlled by quantitative trait loci (QTL) provides effective long-term defense against many pathotypes. The objective of this study was to identify QTL conferring partial resistance against P. sojae from a new genetic source, PI 427106. Ninety-four recombinant inbred lines (RIL) from a F6×8 population of OX20-8 (susceptible) by PI 427106 (with high level of partial resistance) were used in this study. The population was genotyped with approximately 200 SNPs using BeadXpress system and the genetic map was constructed. To evaluate the level of partial resistance, 7-day-old seedlings (10 plants per RIL) were inoculated on the root with P. sojae isolate 13S1.2 and lesion length was measured 7 dai. The mean lesion length of ten seedlings was 8.0 cm. The disease occurrence cause the dramatic decrease in yield and quality of nut production which is required for peritheca maturation. Another ACS coding gene, acs2, has accessory functions for acs1 in most of the physiological processes and has also compensational function for ACL as a nuclear acetyl-CoA producer. Because ACS is a component of pyruvate-acetaldehyde-acetate pathway, this fermentation process might have crucial roles in various physiological processes even for obligate aerobic fungi. In this study, we concluded that acetate is readily generated during the whole life cycle of G. zeae and has central roles for fungal metabolisms.

Functional analyses of two acetyl coenzyme A synthetases in the ascomycete Gibberella zeae

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Phytopathology 101:S100

As acetyl coenzyme A (acyl-CoA) is a crucial metabolite for energy metabolism and biosynthetic pathways, acetyl-CoA should be conserved produced in various cellular compartments spatially and temporally. Our previous study on ATPh citrate lyase (ACL) in destructive plant pathogen Gibberella zeae revealed that ACL-dependent acetyl-CoA generation is important for histone acetylation essential for developmental foci and not for lipid synthesis. We deleted another acetyl-CoA synthetic genes, acetyl-CoA synthetases (acs1 and acs2), to find alternative acetyl-CoA producer for ACL. The acs1 deletion resulted in defect of sexual development partially because of reduced lipid production which is required for peritheca maturation. Another ACS coding gene, acs2, has accessory functions for acs1 in most of the physiological processes and has also compensational function for ACL as a nuclear acetyl-CoA producer. Because ACS is a component of pyruvate-acetaldehyde-acetate pathway, this fermentation process might have crucial roles in various physiological processes even for obligate aerobic fungi. In this study, we concluded that acetate is readily generated during the whole life cycle of G. zeae and has central roles for fungal metabolisms.

Rice chitinase gene contributes to rice sheath blight disease resistance

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Phytopathology 101:S100

Rice chitinases co-localize with disease resistance QTL and are implicated in multiple defense responses. Previous work demonstrated that the class IV rice chitinase Os02g39330 is linked to a disease resistance QTL on chromosome 2, and that this gene is transcriptionally active in response to fungal pathogen attack. We used an RNAi silencing approach to determine if Os02g39330 contributes to broad-spectrum disease resistance. The effect of the silencing construct was measured on expression of Os02g39330 and two closely related chitinases, Os04g41680 and Os04g41620 in five transgenic lines after inoculation with Bipolaris oryzae. Three of the five transgenic lines exhibited high levels of silencing of Os02g39330, and little to no silencing of Os04g41620 and Os04g41680. These lines showed increased sheath blight disease, but less rice blast disease, relative to control lines with no silencing suggests that Os2g39330 contributes to R. solani resistance. Os2g39330 was not associated with M. oryzae resistance in this study. Enhanced expression of related chitinases Os04g41680 and Os04g41620 in the transgenic lines with increased resistance to sheath blight or rice blast, suggesting that these genes do not contribute to disease resistance or susceptibility. The demonstration that Os2g39330 contributes to sheath blight resistance shows that this class IV chitinase is a valuable source of basal resistance for QTL breeding programs.

Management of Phytophthora capsici and potential human foodborne pathogens in irrigation water

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Phytopathology 101:S100

Contamination of surface water with plant pathogens and harmful human pathogens has the potential to reduce crop yield and lower the microbial quality of produce, respectively. In cooperation with local growers, the efficacy of commercial chlorine gas (Cl2) and chlorine dioxide (ClO2) irrigation water injection systems in killing human pathogens and Phytophthora capsici was evaluated. Using predefined parameters, irrigation water treated with Cl2 was effective at reducing populations of coliforms and generic Escherichia coli to below EPA standards for recreational water at all sampling points in 2009, but only at the most distal emitter in 2010. The efficacy of ClO2 in both years was variable with coliform levels never dropping below EPA standards. However, in 2010, generic E. coli populations were at acceptable EPA levels at the most distal emitter. Using baiting techniques in combination with P. capsici-specific PCR, P. capsici was detected in all the local irrigation water sources sampled but not on cucumbers collected from Cl2 or ClO2 treated or non-treated treated water in 2009. Many environmental factors contribute to the efficacy of Cl-based chemicals, including water temperature and pH and organic matter load. Finding ways to reduce the impact of these factors on the effectiveness of these treatments will be critical if such management strategies are to be reliably and sustainably.
Development of a multivariate matrix to trace Clavibacter michiganensis subsp. michiganensis through tomato greenhouse operations

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Phytopathology 101:S101

Clavibacter michiganensis subsp. michiganensis (Cmm) is a seedborne pathogen that spreads rapidly through tomato greenhouse operations, causing significant losses. The genetic diversity of Cmm has been exploited in the past to discern its origin and distribution, but no formal traceability system has been developed. Using geographical information, propagation and production flow diagrams and varietal and seed source data, an industry-specific multivariate matrix that was superimposed with repPCR fingerprints of Cmm strains was designed. The multivariate matrix allows Cmm phenotypic and genotypic information to be recorded and transmitted at any specific point in the production system and the point of origin of each strain can be quickly identified. The efficacy of this system is currently being evaluated and four new Cmm clonal groups have been identified. Producer implementation of the multivariate matrix has the potential to improve production efficiency, improve phytosanitary practices by identifying possible control points in production, decrease disease management related costs and identify new and emerging strains of Cmm. This system will also allow for further advancement of our knowledge of the diversity and distribution of this pathogen throughout North America.

Effect of intermittent leaf wetness on incidence and severity of gray leaf spot of perennial ryegrass turf

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Phytopathology 101:S101

Gray leaf spot, caused by Magnaporthe oryzae, is a devastating disease of perennial ryegrass (Lolium perenne) turf. Free leaf moisture is required for development of the disease. The objective of this study is to determine the effects of intermittent leaf wetness periods on incidence (% leaf blades symptomatic) and severity (Index 0-10; 0 = asymptomatic; 10 ≥ 90% leaf area necrotic) of gray leaf spot of perennial ryegrass turf. Six-weeks-old perennial ryegrass plants were inoculated with M. oryzae (8 × 106 conidia/ml H2O), and maintained at 28°C to allow the disease to develop. Disease incidence and severity were assessed seven days after inoculation. The results showed that there were significant effects of wet/dry periods on disease incidence and severity. Highest disease incidence and severity were recorded on plants exposed to the longest leaf wetness period (18 h continuous leaf wetness). Increased interrupted dry periods significantly reduced gray leaf spot incidence and severity. Additionally, there were negative correlations between interrupted dry period and disease incidence or severity, and the relationships were best described by a quadratic model for disease incidence: Yinc = -5.04X + 0.10X2 + 91.08 and a linear model for disease severity: Ysrev = -0.27X + 9.05 (Inc = Incidence; Sev = Severity; X = dry period). Results of this study may be applied as a component of gray leaf spot disease management strategy in perennial ryegrass fairways in golf course.

Effects of venom alkaloids from red imported fire ants on bacterial canker of tomato in the greenhouse

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Phytopathology 101:S101

Bacterial canker of tomato caused by Clavibacter michiganensis subsp. michiganensis (CMM) has caused major economic losses in tomato production worldwide. This study reported the use of purified venom piperidine alkaloids from the red imported fire ant, S. invicta, to control bacterial canker on tomato seedlings in the greenhouse. Sterilized Redi-Earth Plug & Seedling Mix was used as growth medium. Surface disinfected germinating seeds of Better Boy and DRK7018F1 were sown in containers. Each container has 10 seedlings. The temperature range of the greenhouse was 18–25°C, and the RH was maintained at 60%–90%. Acetone solutions containing 0, 178.56 and 357.12 µg piperidine alkaloids/ml were sprayed twice onto the tomato seedlings at the 4–5 leaf stage with a 7 day interval. Three weeks after the second spray, wounds on tomato leaves and stems were created by gently applying finger pressure, and then a nutrient broth containing 6 × 106 CMM cfu/ml was sprayed onto the tomato plants. Growth containers were then kept at RH 90%-100% for 5 days. Bacterial lesions on the young stems and petioles were investigated 35 days after inoculation. The results indicated that piperidine alkaloids significantly (P < 0.05) reduced the lesion numbers on tomato plants of both Better Boy and DRK7018F1. There was no significant (P > 0.05) difference between the two piperidine alkaloid concentrations. It was observed that DRK7918F1 had higher disease resistance than Better Boy.

Identification of genes involved in biofilm formation using an EZ-Tn5 mutant library of Xanthomonas citri ssp. citri strain 306

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Phytopathology 101:S101

Xanthomonas citri ssp. citri (Xcc) causes citrus canker, one of the most destructive diseases of citrus worldwide. Increasing evidence suggests that the ability to form biofilm is important for host infection by Xcc. However, little is known about the mechanisms of biofilm formation in Xcc. To identify genes involved in biofilm formation by Xcc, an EZ-Tn5 transposon library containing 22,000 clones of Xcc strain 306 were screened in 96-well polystyrene plates, and a total of 292 mutants were selected for biofilm-defective phenotypes. The transposon insertion sites were determined by sequencing analysis combined with random amplifications of transposon ends and homology search of the whole genome sequence of Xcc strain 306. A total of ninety-two genes and five insertions in intergenic regions were revealed to be involved in biofilm formation. Among the 92 disrupted genes, 17 encode translational coupling, which remain only partly understood in this and other systems.
proteins involved in extracellular polysaccharide and/or lipopolysaccharide biosynthesis; 19 others encode proteins involved in flagellum, type IV pili biosynthesis, bacterial chemotaxis or motility; additional 9 encode metabolic enzymes, 4 others for genetic information processing, 2 involved in signal transduction, and 4 with similarity to membrane transporter; In addition, 17 hypothetic proteins with unknown function and 16 not well characterized with putative functions were identified. The genes identified in this study should be helpful for future research into the molecular mechanisms of biofilm formation by Xcc.

Effect of EnvZ/OmpR and GrrS/GrrA systems on Erwinia amylovora virulence
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Phytopathology 101:S102

Erwinia amylovora is a necrogenic enterobacterium causing fire blight disease on rosaceous plants. Early reports have shown that two-component systems in E. amylovora play a major role in virulence and in regulating amylovoran production, including EnvZ/OmpR and GrrS/GrrA, two widely distributed systems in gamma-proteobacteria. While both systems negatively control amylovoran biosynthesis, deletion mutants of envZompR and grrArrs have opposite swarming motility phenotypes. In order to determine how the two systems interact, two triple mutants (envZompRgrrA and envZompRgrrS) were generated. Our results showed that both triple mutants had increased virulence on apple shoots as compared to that of wild type (WT) as well as mutants deleting a single system. In an in vitro amylovoran assay, amylovoran production was significantly increased in the two triple mutants, indicating that the two systems synergistically regulate amylovoran production. In consistency with amylovoran production, amsG gene expression was expressed significantly higher in the triple mutants in vitro than those in WT as well as mutants deleting a single system. Furthermore, the triple mutants showed reduced swarming motility on swimming plates compared to grrArrs mutants and WT strain, but moved faster than envZompR mutants, indicating that the two systems antagonistically regulate swarming motility in E. amylovora.

Identification of species and pathotypes of cereal cyst nematode in winter wheat on the Huang-Huai floodplain of China
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Phytopathology 101:S102

The cereal cyst nematode (CCN) of wheat has become a severe disease problem in the winter wheat on the Huang-Huai floodplain, the biggest bread basket of China. Heteroderid specimens at 21 locations in Henan and adjacent provinces were identified by morphological and molecular analysis. H. filipjevi was found in six locations in Henan, including two mixed with H. avenae; H. avenae type “C” being found at all other locations. Thirteen CCN populations consisitng of four populations belong to H. filipjevi and nine belong to H. avenae were typed using 23 standard international differentials (13 group A for main test and 10 group B for assistant test) and a common local cultivar Wemenai 19. These populations were found to be previously undescribed pathotypes. There are 4 different pathotypes for 4 H. filipjevi populations from Henan province, with the different virulence to 23 differential hosts. Three populations of H. avenae of the pathotype from Xushui and Xingyang were typed for group 3, which were virulent to Ha1 and Ha2 genes in barley, but avirulent to Ha3 gene; Other 7 populations were divided into group 1 pathotype, which were avirulent to Ha1, Ha2 and Ha3 genes. The resistance reaction of 13 group A differentials hosts to 4 populations from Anyang, Qingfeng, Heze and Yangshang was same, but different from Haidan, Baoding and Shangju populations, being by avirulent in wheat cv. Iskomani-K-2 (Haidan population) or barley cv. KVL191 (Baoding and Shangju population).

Simultaneous detection and differentiation of four sweet potato potyviruses by one-step RT-PCR
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Phytopathology 101:S102

At least 20 viruses are reported to infect sweet potato and cause serious yield losses worldwide, and six of them are species of the genus Potyvirus in the family Potyviridae. Sweet potato feathery mottle virus (SPFMV), Sweet potato virus C (SPVC), Sweet potato virus G (SPVG) and Sweet potato virus Y (SPVY), three newly recognized viruses. Identification and detection of these viruses is complicated by high similarity among their genomic sequences, frequent occurrence as mixed infections and low titer in many sweet potato cultivars. A one-tube quadruplex reverse transcription (RT)-PCR assay was developed for the simultaneous detection and differentiation as SPFMV, SPVC, SPVG and SPVY. Four virus-specific forward primers and one reverse primer based on the region conserved in all four viruses were selected. The assay was optimized for primer concentration, cycle number, conditions of annealing and elongation steps. The assay was first tested using a sweet potato plant naturally infected with all four viruses, and then evaluated using other single- and mix-infected plants in our collection and field samples from southwestern China. This RT-PCR is reliable and sensitive as a simple, rapid and cost-effective method to detect these pathogens in sweet potato. The assay will be useful to quarantine and certification programs as well as virus surveys when large numbers of samples need to be tested.

Phylogenetic relationships of closely related potyviruses infecting sweet potato determined by genomic characterization of Sweet potato virus 2 and Sweet potato virus G
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Phytopathology 101:S102

Several closely related potyviruses including Sweet potato virus 2 (SPV2) and Sweet potato virus G (SPVG) are reported to cause disease in sweet potato, and their classification is confusing. In this study, complete nucleotide sequences of a SPV2 isolate and two SPVG isolates were determined to be 10,732 and 10,800 nucleotides, respectively, excluding the 3'-poly(A) tail. Their genomic organization is typical of potyviruses, encoding a polyprotein with an N-terminal genome-linked protein (Gp1) and then a series of viral proteins non-reading frame encoded from frame 2. The polyprotein is polyprotein-coded as 17 individual structural and non-structural proteins. Phylogenetic comparisons of the viral sequences and deduced polyprotein and individual protein sequences of the two viruses with those of 72 other potyviruses confirm that both SPV2 and SPVG are distinct species of the genus Potyvirus in the family Potyviridae. Phylogenetic trees constructed from the genomic, polyprotein sequences show that SPV2 and SPVG are most closely related to each other, and they form a distinct clade with Sweet potato feathery mottle virus (SPFMV) and Sweet potato virus C (SPVC). Phylogenetic analysis based on the deduced amino acid sequences of the 3'-terminal genome without a partial N terminus and C terminal signals suggests that the viruses in this clade can be divided into five species, SPFMV, SPVC, SPVG, and Sweet potato virus -Zimbabwe. The analysis also reveals that Sweet potato virus G and Ipomoea vein mosaic virus are grouped with SPV2 as one species, and show these two viruses should be consolidated as SPV2.

454-Pyrosequencing reveals the influence of organic and conventional farming systems on beneficial bacterial communities to enhance plant health
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Phytopathology 101:S102

Bacterial communities in soil exert versatile functions in maintaining soil and plant health. We investigated the long-term impacts of crop rotation on the beneficial bacterial communities to enhance plant health. Soil samples were collected from Canada’s oldest organic-conventional study Glenlea, Manitoba. The main plots were two crop rotations; fallow-fababean-wheat (grain only) and wheat-alfalfa-alfalfa-flax (grain forage), and certificated organic and conventional methods served as subplots. A total of 125,316 sequences were generated. A total of 14 phyla were represented in the dataset, with Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, and Firmicutes forming the most dominant phyla. Proteobacteria was significantly high under grain only organic system (44.45%), while it was only 27.25% under the forage grain conventional farming system. Actinobacteria were 43.12% under the forage grain conventional system, while grain only organic system had a lower percentage (32.48%). When bacteria were analyzed at the genus level, the relative abundances of different genera belonging to phyla Actinobacteria and Proteobacteria varied among the samples under different treatments. The genus belonging to Proteobacteria, such as Pseudomonas, Stenotrophomonas, Brevundimonas, were more frequently found in organic farming systems. Pyrosequencing revealed beneficial soil bacterial communities shifts resulting from different cropping systems that could have an impact on plant health.

Pathogenic Ebellmisia astragali on Astragalus absurdes is very closely related to locoweed endophyte
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Phytopathology 101:S102
Embellisia astragali which resides within Astragalus adsurgens is a pathogenic fungal species recently identified in China. Its conidia, colony morphology and growth rate are similar to that of the locoweed endophyte, Undifilum oxytropis. The two fungi were compared using morphology and genetics. DNA of both fungi was assessed using a pair of Undifilum-specific primers, OR1 and ITS5 which amplify a portion of the ITS region. The amplicons from both fungi were tested for digestion with the restriction endonuclease AclI close genetic relationship.

A new Botrytis sp. causing grey mold on blackberry

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Phytopathology 101:S103

Botrytis spp. cause blossom and fruit diseases on many crops, including blackberry. In a survey of 4 locations in the state of South Carolina, 85 isolates of Botrytis spp. were collected from blackberry fruit and single spores were characterized. Two distinct species, B. cinerea and another undescribed species, were identified based on examination of the non-coding ITS region and the coding G3PDH, HSP60 and RPB2 genes and morphological characters. The new species formed pale yellow to white colonies with short aerial mycelia and produced black sclerotia on PDA at 20°C. Phylogenetic analysis based on combined DNA sequence data of three nuclear genes (G3PDH, HSP60 and RPB2) showed that the novel Botrytis sp. is most closely related to B. fabiopsis, the causal agent of gray mold disease of broad bean, and B. galantina. Its conidia, however, are smaller than conidia of B. fabiopsis and B. galantina and sequence analysis of genes encoding nectrosis and ethylene-inducing proteins (NEPs) also indicated that the novel Botrytis spp. is distinct from B. fabiopsis. The new species is pathogenic on broad bean leaves, which distinguishes it from B. galantina. Inoculation of blackberry fruit with conidia caused typical gray mold symptoms but compared to B. cinerea the latent period was significantly longer. In conclusion, we discovered a new species of Botrytis on blackberry which is potentially pathogenic on other crops judged by its pathogenicity on broad bean leaves.

Identification of soybean accessions with resistance to Phomopsis seed decay: Joint effort from USDA and University scientists

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Phytopathology 101:S103

Soybean Phomopsis seed decay (PSD) is primarily caused by Phomopsis longicolla along with other Phomopsis and Diaporthe spp. This disease causes poor seed quality and suppresses yield in most soybean-growing states in the United States. In 2009, PSD caused yield loss of over 12 million bushels in 16 southern states. To identify new sources of resistance to PSD, seed of 208 representative maturity group V soybean lines, obtained from the USDA Soybean Germplasm Collection in 2006, were plated and assayed for the percentage of Phomopsis seed infection. Based on the results of seed assays from 2006 and 2007, 14 accessions were selected for further evaluation with inoculated and non-inoculated treatments in 2008 and 2009. In addition, 135 soybean cultivars from major growing areas in the USA were field screened by natural infection in 2009 at Kibler, AR, Stoneville, MS and Portageville, MO. Based on the seed assay in 2009, 42 lines along with six resistant and susceptible checks were selected and field-tested with inoculated and non-inoculated treatments in these states in 2010. In 2009, frequent rainfall during seed maturation led to high levels of seed infection by Phomopsis (up to 80%) and other fungal pathogens for most soybean lines but several lines were identified that had low percentage of seed infection, good visual quality, and high germination rates. These resistant sources will be used to develop cultivars resistant to PSD.

Identification and virulence differentiation of Colletotrichum gloeosporioides, the causal agent of grapevine anthracnose in China

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Phytopathology 101:S103

Grape anthracnose become more and more serve in many of warm and moist regions in China these years. Disease samples were collected from Beijing and other 6 provinces, and 135 isolates were pured. On the characterization of colony, conidia, acervulus and rDNA-ITS sequences, the isolates were identified as Colletotrichum gloeosporioides (Penz.) Sacc. With the characterization of colony, sporation, mycelium growth and pathogenicity to 14 grapevine cultivars, the isolates were classed to three distinct virulence phenotypes, Group I, Group II and Group III, the ratios are 33.75%, 13.75% and 52.2% respectively. Three representative isolates belongs to different groups were used to analysis with 64 pairs of AFLP primer. There are 1059 bands in the gel and 489 are polymorphism bands, the polymorphism ratio is 32.41%. The results shown that there are some differences between the different groups in molecular level. Then, 20 random isolates were selected to identify molecular markers linked to different groups, at last, the primer E13/M14 were isolated, and the similarity is 85% between phenotypes and AFLP marker.

Use of an integrated system for disease monitoring and forecasting of wheat stripe rust in China

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Phytopathology 101:S103

Optimization of management resources at national level and maximization of beneficial returns from control measurements are top priorities in the national management program of wheat stripe rust in China. In 2010 spring, a new disease forecasting system based on mid-term weather forecasts and regional disease modeling approaches was incorporated into the current disease monitoring system. The integrated system can predict long distance spore dispersal and a weather favorability of disease development. The forecasting is driven by modeled daily weather data and field monitoring disease data updated weekly. Forecasting results are mapped to show weekly spore dispersion and monthly weather favorability to show the disease risks to grain growers, Extension specialists and Agent counselors. In the season, the forecast system was successful in predicting several localized occurrences in several regions so that timely control was taken to prevent disease build-up. Field surveys in the late season showed that the disease was at low level nationwide as predicted.

Volatile organic compounds produced by Ceratocystis fimbriata and their inhibition on plant pathogenic fungi

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Phytopathology 101:S103

Ceratocystis fimbriata is the pathogen of Pomegranate Wilt which occurred in Mengzi country of Yunnan province China recently and first reported in 2003. It has been indicated that C. fimbriata has the ability of generating a complex variety of aromas and distributed in the soil. Volatile organic compounds (VOCs) can be identified and quantified by headspace GC-MS. This study revealed that headspace analysis is a perfect way to detect the VOCs production from C. fimbriata strains isolated from infected pomegranate and sweet potato. Ten compounds, of which ethanal, aceticacidbutylester and acetaldehyde were the most abundant aroma volatiles, and carbon disulfide was first reported. The production of total volatile reached 6660.86 ng/mL. By means of dual test and vertical dual test in vitro without physical contact or diffusion through the culture medium, it was shown that VOCs produced by C. fimbriata inhibited significantly the growth of experimental target fungi, including Botrytis cinerea, Monilinia fructicola, Falsa mali, Fusarium oxysporum, Fusarium sp. and Curvularia. There was an increasing inhibitory activity as the culture time lasting longer with more volatile compounds produced by C. fimbriata. The fruity flavour also affected pigments production of Fusarium. Construction of VOCs-C. fimbriata bioreactor will be a potential method for controlling plant diseases.

The synergy between Bombyx mori gut bacteria and insecticidal crystal protein of Bacillus thuringiensis to its larvae

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Phytopathology 101:S103

In recent years, some researchers began to study the roles of gut bacteria on insecticidal activity of Bacillus thuringiensis (Bt), and put forward new evidences and views that gut bacteria of insect host have synergistic or antagonistic effect, it may promote pest control. This work stated the roles of gut bacteria on insecticidal activity of Bt in Bombyx mori, providing a foundation to illustrate the roles of gut bacteria and insecticidal mechanism of Bt. Presently, we will discuss the following progress. Seven dominant bacteria were identified their characteristics preliminarily. Bioassay showed Cry toxin can be lethal to sterile larvae, but its value of LC50 was higher 4 times than normal larvae approximately. This indicated that the intestinal bacteria are not necessary for Bt insecticide, but the intestinal bacteria may play a synergistic
role. The intestine of intestinal flora to Cry toxin may be related to species-specific. Through comparing bioassay, we found the intestinal bacteria MB1 synergize to Cry toxin significantly. We found the MB1 played a role in accelerating the activation of protection. In addition, MB1 still led a higher mortality than the control. Therefore, MB1 have a complex impact on the pathogenicity of larvae.

Protein extraction methods and proteomic analysis of the locoweed filamentous fungus Undifillum oxytropis

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Phytopathology 101:S104

Locoism, caused by swainsonine, is a serious disease around the world. Swainsonine is synthesized by the fungus Undifillum oxytropis in locoweeds, as well as the whitefly Bemisia tabaci, which causes toxicosis and severe economic losses in the western United States and China. Details about how swainsonine is synthesized by U. oxytropis endophyte, and the interaction between the fungus and locoweeds are poorly understood. Information the U. oxytropis proteome could be particularly valuable to help address the problem. Protein sample preparation is critical and challenging for two dimensional gel electrophoresis for protein analysis. Unfortunately, there is no single protein extraction method that can be universally applied to all kinds of organisms analysed by 2-DE. To develop an optimized protein extraction protocol for U. oxytropis, five protein extraction methods were evaluated. To our knowledge the present study is the first proteome analysis using 2-DE for U. oxytropis, and the resolution of the 2-DE reference map is a useful approach for proteomic analysis. This proteome map aided identification of putative consumption of locoweeds causes significant livestock poisoning and severe economic losses in the western United States and China. Screening several hundreds of AMT transformants identified three stable mutants that showed significantly reduced virulence/pathogenicity factors in S. sclerotiorum. Random mutagenesis via random mutagenesis retain pathogenicity X. LIANGSHENG (1), M. Xiang (1), D. White (1), W. Chen (2)
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Phytopathology 101:S104

Sclerotinia sclerotiorum is a ubiquitous necrotrophic plant pathogen capable of infecting over 400 plant species including many economically important crops. Oxalic acid production has been shown in numerous studies to be a pathogenicity factor for Sclerotinia sclerotiorum through several mechanisms. Random mutagenesis through Agrobacterium-mediated transformation (AMT) was used to study pathogenic mechanisms in Sclerotinia sclerotiorum. Screening several hundreds of AMT transformants identified three stable mutants that were unable to produce oxalic acid. The mutants did not lower pH of agar plates, and no oxalic acid was detected in liquid medium or in mycelium of the mutants using HPLC. However, the oxalate-minus mutants showed similar levels of virulence comparable to the wild type strain in colonizing pea leaves in detached leaf assays. Southern hybridization blots showed the mutation was due to a single T-DNA insertion and the T-DNA insertion site was identified to be located in the gene of oxaloacetate acetylhydrolase of S. sclerotiorum. The results showed that oxalic acid is not required for pathogenicity of Sclerotinia sclerotiorum.

Random T-DNA mutagenesis identifies a Cu-Zn-superoxide dismutase gene as a virulence factor of Sclerotinia sclerotiorum

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Phytopathology 101:S104

The Ascomycetous fungus Sclerotinia sclerotiorum is a devastating pathogen capable of infecting more than 400 plant species including many economically important crops. In order to gain a better mechanistic understanding of its non-specific host-pathogen interactions, random mutagenesis through Agrobacterium-mediated transformation (AMT) was used to identify potential virulence/pathogenicity factors in S. sclerotiorum. Screening several hundreds of AMT transformants identified two stable mutants that showed significantly less virulence in comparison with the wild type strain as measured by colonizing pea leaves in detached leaf assays. Southern hybridization analysis showed that the mutation was due to a single T-DNA insertion, and inverse PCR methods (T-DNA end site approach) identified that the T-DNA insertion site was in the gene of the Cu-Zn-superoxide dismutase (SOD, SS1G00699) of S. sclerotiorum. This SOD gene consists of an open reading frame of 465 bps, and its expression levels were significantly induced under oxidative stresses or during infection of pea plants. These results suggest that this SOD gene plays critical roles in detoxification of reactive oxygen species during host-pathogen interactions and is an important virulence factor of S. sclerotiorum in pathogenesis.

Evidence of genetic diversity and heterothallism in Sclerotinia homoeocarpa, the causal agent of dollar spot disease on turfgrass

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Phytopathology 101:S104

Sclerotinia homoeocarpa F.T. Bennett causes dollar spot disease (DSD) on warm season and cool season turfgrasses in Florida and in northern states. The morphological and genetic relationship among DSD isolates collected from warm and cool season turfgrasses is unclear. We characterized a collection of 47 isolates of S. homoeocarpa from both warm and cool season turfgrass species in Florida and northern states. Screening the collection for morphological characters showed that the Florida collection mainly was represented by a novel strain of S. homoeocarpa. We found differences in mycelia pigmentation, stroma formation, and symptom development on St. aggregata, a warm season vegetable when compared to strains from the Florida and northern strains. We characterized and sequenced the MATI-2 high-mobility group and MATI-1 a box mating-type gene loci from the collection. Our data demonstrate an idiomorphic structure and an equal distribution of mating types in our collection characteristic of out crossing heterothallic fungi. Phylogenies established with the MAT locus and variable regions of the ribosomal DNA provided additional support for the observed morphological distinction between the strains. These findings provide new insight into the genetic diversity of this pathogen and its geographic distribution.

Malvaviscus yellow mosaic virus, a weed-infecting begomovirus carrying a nanovirus-like nonanucleotide and a modified stem-loop structure

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Phytopathology 101:S104

Begomoviruses (family Geminiviridae) have a circular, ssDNA genome encapsidated in twinned icosahedral particles. In Brazil, a number of begomoviruses infecting weeds have been described, and evidence suggests that they have given rise to the viruses currently found in crop plants. Here we describe a novel begomovirus infecting a Malvaviscus arboresus plant showing a bright yellow mosaic, collected at the experimental farm of the Campinas Agronomical Institute (IAC), in Campinas, Brazil, in May 2005. Total DNA was extracted and the viral genome was amplified by RCA, cloned and sequenced. Sequence analysis indicated that the virus corresponds to a novel species, for which the name Malvaviscus yellow mosaic virus (MalYMV) is proposed. Strikingly, MalYMV has a nanovirus- like alphasatellite-like nonanucleotide (TAGTATTACC). Moreover, a short sequence located 5' of the nonanucleotide forms a minor hairpin structure embedded in the major hairpin. Nevertheless, the loop where the nonanucleotide is located is similar in size to the conserved begomovirus structure. The relevance of this modified structure is unknown. Although MalYMV has been collected in Brazil, it is phylogenetically close to viruses from Central and North America. The M. arboresus plant has been displaying the observed yellow mosaic symptoms since at least the 1960's (as noted by the sixth author), which suggests that MalYMV may be poorly transmitted (or not transmitted at all) by local whitefly populations.

Discovery the new synthesized of PTGS-related small RNAs by an ultrasensitive silicon nanowire field-effect transistor and Next-Generation Sequence

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Phytopathology 101:S104

Post-transcriptional gene silencing (PTGS) is an antiviral strategy of the plant to avoid the virus infection. However, the virus produces a viral suppressor to suppress the PTGS for preventing the silencing machinery to attack the virus. P19 of Tombusvirus (19 kDa) has been identified as a viral suppressor and specifically binds 21 nt double-stranded siRNA (ds-siRNA). In this study, we combined the nanotechnology and the RNA binding ability of p19 protein on silicon nanowire field-effect transistor (SiNW-FET) probe. This p19-SiNW-FET sensor has very high sensitivity to detected 21 nt ds-siRNA. Besides, the sensor has an ability to distinguish the various secondary structures of ds-siRNA, such as the size or mismatch on the ds-siRNA. The ds-siRNAs that bound on the p19-SiNW-FET probe. This p19-SiNW-FET sensor has combined the nanotechnology and the RNA binding ability of p19 to develop specifically binds 21 nt double-stranded siRNA (ds-siRNA). In this study, we combined the nanotechnology and the RNA binding ability of p19 protein on silicon nanowire field-effect transistor (SiNW-FET) probe. This p19-SiNW-FET sensor has very high sensitivity to detected 21 nt ds-siRNA. Besides, the sensor has an ability to distinguish the various secondary structures of ds-siRNA, such as the size or mismatch on the ds-siRNA. The ds-siRNAs that bound on the p19-SiNW-FET probe. This p19-SiNW-FET sensor has combined the nanotechnology and the RNA binding ability of p19 to develop
DS-siRNA were belong to the new-synthesized siRNA by PTGS pathway whereas some species of siRNAs were unable to detect in the input of total small RNA profile. The SiNW-FET combined with NGS technology provides a good strategy to study the protein-RNA interaction between PTGS and viral suppressor and also able to discover the new synthesized PTGS-related DS-siRNAs in the plant.

Genotypic classification of pathogenic variants of Xanthomonas axonopodis pv. citri from Taiwan by various DNA typing methods

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Phytopathology 101:S105

Molecular typing was applied for genotypic classification for three pathogenic variants of Xanthomonas axonopodis pv. citri (Xac) from Taiwan. These three novel variants of atypical symptom-producing Xac were designated as Xac-A’, -Ap and -Ar. Based on the polymerase chain reaction (PCR) with primers specific to Xac, leucine-responsive regulatory protein (lrrP) gene assay and DNA fingerprintings generated by repetitive-sequence PCR (rep-PCR) and amplified fragment length polymorphism (AFLP) were optimized to compare strains including the three types of atypical symptom-producing strains Xac-A’, -Ap and -Ar, and additional reference strains from pathotypes Xac-A, -Ap, -Ar, X. axonopodis pv. aurantifoli and X. axonopodis pv. citrumeelo. These three types of Xac variants could be detected with six sets of primer specific for Xac. Cultor clusters by lrrP sequence assay, AFLP and combing the band patterns of rep-PCR clearly grouped these variants in types Xac-A’, -Ap and -Ar into the same cluster with typical symptom-producing strains in pathotype Xac-A. These three types of Xac variants could be excluded from strains of Xac-A* and -A* in these genotypic analyses. Strains of Xac-A* and -A* were closely related to Xac-A strains in our results. NoTaiwan isolate was related to X. axonopodis pv. aurantifoli or X. axonopodis pv. citrumeelo. The results further confirm the atypical symptom-producing variants of Xac in Taiwan belong to pathotype Xac-A.

One-step multiplex RT-PCR assay for simultaneous detection of two viroids and Plum bark necrosis stem pitting-associated virus in stone fruit trees

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Phytopathology 101:S105

Hop stunt viroid (HSVd), Peach latent mosaic viroid (PLMVd) and Plum bark necrosis stem pitting-associated virus (PBNSPaV) infect stone fruit trees. A single-step multiplex RT-PCR assay (mRT-PCR) was developed for the simultaneous detection of these pathogens. Three pairs of primers were designed to yield pathogen-specific amplicons, and amplification of a plant 185 rRNA fragment was included as a control. The expected products of 400 bp (PBNSPaV), 297 bp (HSVd), and 207 bp (PLMVd) were amplified from plants infected with each pathogen by both uniplex and mRT-PCR. The sensitivity and specificity of mRT-PCR was similar to that of the uniplex RT-PCR. The method was validated using samples from our collections that included single trees infected with one, two or three of the pathogens, as well as samples from the National Clean Plant Network. HSVd, PLMVd and PBNSPaV were detected from a co-infected peach tree, and both HSVd and PBNSPaV were detected from a co-infected myrobalan plum tree. The mRT-PCR was also evaluated by testing field samples from four different sources, and from these tests both PLMVd and PBNSPaV were detected as single infections. This assay is reliable, sensitive, and is a rapid and cost-effective method to detect these pathogens in stone fruit trees. The procedure is useful for certification, quarantine and genebank programs that test germplasm before distribution, and is especially applicable when many samples are tested for all three pathogens.

Development of a real-time RT-PCR assay to detect Peach latent mosaic viroid infections in stone fruit trees

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Phytopathology 101:S105

Peach latent mosaic viroid (PLMVd) infects Prunus and Prunus species. It is distributed worldwide and each cause economic damage through reductions in fruit quality and yield. A single tube real-time TaqMan RT-PCR assay was developed for the detection of PLMVd, and compared to a conventional RT-PCR assay. Total nucleic acids were extracted from healthy and infected fruit trees using a CTAB method. The assay included a fluorogenic cytochrome oxidase gene probe (COX) as an internal control to validate the quality of the total RNA template samples. The assay was evaluated using PLMVd isolates collected from different geographic origins and field samples from commercial orchards in Colorado. The results of the TaqMan RT-PCR correlated with those from conventional RT-PCR and the sensitivity of this assay was 10⁻⁷, a 10-fold increase in sensitivity over the conventional assay. This assay could be useful as a fast and sensitive option for PLMVd detection in quarantine and certification programs. It decreases the risk of contamination by performing the entire test in a single tube. It also requires less reaction time and avoids post RT-PCR electrophoresis, which reduces the labor involved when a large number of samples are tested.

The effect of Potato virus S infection on late blight severity in selected potato genotypes

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Phytopathology 101:S105

Late blight caused by Phytophthora infestans is an important disease of potato. cv. Defender is the only cultivar with foliar resistance to late blight. However, this cultivar exhibited susceptibility to infection by Potato virus S (PVS). PVS infection is very common in commercial potato fields in the Columbia Basin of Pacific Northwestern U.S.A. To investigate the potential interactions between these two pathogens and the resulting response of various potato genotypes, ‘Defender’ and Ranger Russet were inoculated with both P. infestans and PVS. The amount of sporulation and the extent of lesion expansion on inoculated leaves were measured to estimate the incidence of late blight. ‘Defender’ showed restricted spot lesions and had twenty times less amount of sporangia compared to ‘Ranger Russet’ when inoculated with P. infestans only. However, lesion expansion and sporulation increased significantly when ‘Defender’ was infected with PVS followed by inoculation with P. infestans. The increased late blight in PVS-infected ‘Defender’ suggests potential interactions between PVS and Defender impacting the outcome to late blight infection.

Complete genome sequence analyses and functional predictions for ‘Ca. L. solanacearum’, the bacterium associated with potato zebra chip disease

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Phytopathology 101:S105

Zebra Chip (ZC) is an emerging plant disease that causes the decline of potato shoots and generally results in unusable tubers. ZC is characterized by a patterned discoloration within the tuber and fried chips from ZC-diseased tubers are commercially unacceptable. The disease has significantly impacted the U.S. potato industry and potato growing regions around the world. ZC is associated with ‘Candidatus Liberibacter solanacearum’ (Lso), a fastidious alpha-proteobacterium that is transmitted by a phloem-feeding psyllid vector, Bactericera cockerelli Sulc. Taxonomically, Lso is related to ‘Ca. Liberibacter asiaticus’ (Las), the putative causal agent of citrus huanglonging. Research on this disease has been hampered by the fact that the bacterium is unculturable, making it impossible to obtain pure bacterial cell-free DNA. In spite of these limitations, high quality genomic DNA was obtained using an immuno-capture technique. The complete 1.26 Mbp metagenome sequence of Lso was determined based on Lso DNA isolated from potato psyllids. The coding inventory of the Lso genome was analyzed and compared to other bacteria within the Rhizobiaceae family to identify genes and predict possible physiological functions. The analyse revealed a number of unique transporters and metabolic pathways, all potentially contributing to ZC pathogenesis. Information derived from this study will facilitate development of effective strategies for controlling ZC disease.

Proteomic analysis of grapevines in response to Xylella fastidiosa infection

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Phytopathology 101:S105

Xylella fastidiosa (Xf) is the bacterial causal agent of Pierce’s disease (PD) of grapevines, as well as of other economically important diseases in a number of agronomic, horticultural and ornamental plants. The objective of this research was to tentatively identify proteins that are expressed in grapevines and involved in disease development or defense responses to Xf infection. A combination of analyses were utilized to identify proteins differentially expressed in Xf-infected grape stems from a pair of siblings of 9621-67 (highly susceptible) and 9621-94 (highly resistant) from a cross of Vitis rupestris × V. arizonica. Total proteins were isolated from the stems of uninoculated and Xf-inoculated plants at 1, 6, and 12 weeks after inoculation, separated by a 2-D PAGE system, and spots representing differentially expressed proteins were analyzed and identified using LC/MS/MS. Results revealed that differential expression of proteins in response to Xf-infection were genotype and development stage dependent. This study provides the first
proteomic analyses of the host responses to Xf infection in highly resistant and susceptible genotypes of grapevines. The information obtained will aid in the understanding of the mechanisms related to the host-pathogen interactions involved in PD.

Deep sequencing of small RNAs for virus and viroid identification in tomatoes

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Phytopathology 101:S106

Viroids are the smallest (246-401 nt) self-replicating plant pathogens. Recent evidence has led to the emerging view that RNA silencing has a crucial role in viroid pathogenesis and evolution, but the small RNA (sRNA) upon viroid infection on tomato plants has not been thoroughly analyzed. The objective of the present study was to conduct deep sequencing of sRNAs in tomato and to compare the sRNA profiles upon infection by Pepino mosaic virus (PepMV) and three poospiviroids, Potato spindle tuber viroid (PSTVd), Tomato chlorotic dwarf viroid (TCVDv), and Mexican papita viroid (MPVd). sRNA libraries were prepared and sequenced using Illumina technology. Libraries were generated from four samples suspected to be infected by PSTVd, TCVDv, MPVd or PepMV. Sequencing produced from 4.8 to 6.7 million reads per library. In all four libraries, sRNAs 23 nt and 24 nt in length were most abundant, followed by 21 nt and 22 nt. Nearly 90% of the sRNA reads in each sample could be aligned to the tomato genome. The reads that did not align to the tomato genome were assembled using previously characterized PepMV and viroid genomes as scaffolds. Results showed that virus or viroid-derived sRNAs from tomato were enriched and extended over respective virus or viroid genome. Greater genetic diversity of these pathogens in field samples was also unveiled. The success of the deep-sequencing of sRNA lends itself not only to virus discovery, but also to identification of unknown viroids.

Evaluation and adaptation of CANARY technology for rapid detection of plant pathogens

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Phytopathology 101:S106

CANARY is a cell-based technology which has been applied in medical diagnostic assays and monitoring for select agents. In this study, we evaluated the method for rapid detection of Ralstonia solanacearum and Phytophthora spp. using B cell lines developed by collaborators at MIT. We determined that the analytical sensitivity of Ralstonia CANARY is comparable to that of a typical PCR-based assay. During the study, we observed that cross-reactivity occurred between B cell lines and non-target pathogens or healthy plant extracts. We discovered that a major type of cross-reactivity resulted from an inherent antibody (Ab) anchored on the outer membrane of the cell. In the Ralstonia assay, the cross-reactivity was completely abolished through a chemical Ab blocking step while maintaining the target CANARY activity. The cross-reactivity between the B cell line and plants was greatly reduced or eliminated by simple sample manipulations. In the Phytophthora assay, chemically blocking the interfering Ab eliminated all CANARY activity, which suggested that the B cell line selected for testing contained only the inherent antibody (Ab) targeting Phytophthora. We re-screened Phytophthora B cell clones consisting of the recombinant DNA of the desirable Ab and identified a clone generating high CANARY activity when the cross-reactivity was blocked. We will present CANARY protocols that maximize the analytical sensitivity and minimize the assay interference.

Air sampling of three powdery mildew populations using a Burkard cyclone sampler in eastern Washington

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Phytopathology 101:S106

Powdery mildews, caused by Erysiphe necator, Podotaphera clandestina, and P. macularis, respectively, are the most important fungal diseases of wine grapes, sweet cherries, and hops in eastern Washington. A reliable, user-friendly air-sampling device is critical for monitoring population pathways, which could aid in ipmology studies and facilitate implementation of disease control measures. A Burkard multivial cyclone sampler was evaluated for studying powdery mildews on the three aforementioned crops in 2010. The multivial cyclone, Burkard volumetric, and Rototod spore traps, were installed adjacent to a vineyard, cherry orchard, and hop yard. The Burkard cyclone and volumetric samplers were programmed to collect samples daily whereas sampling medium was changed weekly for Rotodot samplers. Samples from the multivial cyclone and Rototod traps were subjected to DNA extraction for amplifying DNA of three pathogens using their corresponding specific real-time PCR assay. Cp values of PCR amplifications were correlated to the log transformed daily spore count data of Burkard volumetric samplers. The correlation coefficients (r) were –0.86 (P < 0.0001), –0.88 (P < 0.0001), and –0.52 (P < 0.0001) for powdery mildews on cherry, grape, and hop, respectively. The Cp values indicated that the cyclone sampler is more efficient for trapping spores. These data indicate that the cyclone sampler shows promise as a reliable device for collecting airborne plant pathogen spores for subsequent PCR detection and quantification.

Biodegradation of cypermethrin by Rhodopseudomonas palustris GJ-22 isolated from contaminated sludge

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Phytopathology 101:S106

Abstract: GJ-22, a strain of the photosynthetic bacterium (PSB) capable of degrading cypermethrin (CPM), was isolated from the sludge from the wastewater treatment unit of an insecticide factory. The strain showed relatively high CPM degradation ability and was identified as Rhodopseudomonas palustris on the basis of its culture characteristics, colony and cell morphology, living cell absorption spectrum analysis, physiological and biochemical characteristics, type of internal photosynthetic membrane, and 16S rRNA sequence similarity analysis. Single-factor tests showed that CPM degradation by GJ-22 was good from 25°C to 35°C, but that the optimal temperature was 30°C; a pH of 7.0 was optimal for both initial growth and CPM degradation. GJ-22 completely transformed CPM at a concentration of 100 mg/L in 50°C, pH 7.0, and 7,500 lux within 7 days. Under optimal conditions, with a week, GJ-22 degraded 83.45% of CPM, 77.09% of fenpropatrin, 71.21% of bifenthrin, and 31.10% of ethenofprox at concentrations of 100 mg/L. The metabolic products were detected by performing gas chromatography/mass spectrometry (GC/MS) analysis; the analysis showed that GJ-22 oxidatively degraded CPM, yielding 5 metabolites. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Antagonism between Trichoderma harzianum ETS 323 and Botrytis cinerea associated with L-phenylalanine oxidase-induced reactive oxygen species generation

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Phytopathology 101:S106

Previous proteomic studies showed that a homogenized L-phenylalanine oxidase (L-PhAO) oxidizing L-phenylalanine (L-Phe) into L-phenyllactic acid (L-PLA) was identified from extracellular proteins of Trichoderma harzianum ETS 323 in the presence of deactivated B. cinerea. Antagonistic assay showed that treatment with purified Th-L-AAO effectively inhibited B. cinerea hyphal growth and caused hyphal swelling, deformed hyphae and vacuolation within hyphae, subsequently leading to hyphal lysis. Interestingly, Th-L-AAO treatment enhanced endogenous reactive oxygen species generation. Further, we evidenced condensed chromatin and DNA fragmentation, increased lipid peroxidation activity, and dissipated mitochondrial membrane potential (∆Ψm), supporting the apoptotic process in B. cinerea.

Protein photocleavers chrysophanol and pachybasin involved in Trichoderma’s biocontrol mechanism

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Phytopathology 101:S106

Anthraquinones have shown activity against phytopathogens, but the mechanism of action is not yet well understood. This study investigated the photocleavage activity on proteins of two anthraquinone derivatives, chrysophanol and pachybasin, isolated from Trichoderma harzianum SY.
Study on the extraction, purification and chemical structure of the activity component from Gymnascus reessii za-130
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PEOPLES REP OF CHINA
Phytopathology 101:S107

The vegetable disease caused by root knot nematode were losing seriously and difficult to control with chemical. Applying bio-materials to control this disease are more safety. The strain of Gymnascus reessii za-130 has ability to kill the root knot nematode (Meloidogyne incognita) and has been reported the result of the nematocidal activity from its broth filtrate and physical-chemical character in 2010. This report was studied on the extraction and purification for activity component of za-130 which including pretreatment of broth filtrate, macroporous absorption and adsorption resin to remove excess impurities, and finally 100% methanol elution, collected and evaporated to dryness; With chloroform: methanol: water dissolving, the concentrated broth filtrate, macroporous absorption and adsorption resin to remove excess.

Isolation, identification of scutellaria extraction by 80% ethanol and its antifungal mechanism against Monilinia fructicola
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Phytopathology 101:S107

Brown Rot caused by Monilinia fructicola is a serious disease for peach trees. Recently, there are some studies showed that the traditional Chinese herbs could have very good anti-fungi effect. However, the inhibition mechanism and activity component are still not clear. Scutellaria is one of Chinese herbs which used for antifungal. In this work, a new compound which could inhibit the growth and sporulation of M. fructicola was isolated and identified. The antifungal mechanism was studied. The growth of the hyphae were significantly thicker under microscope, and conidia can’t be formed under the same conditions. No remarkable difference was found between the antifungal activities of the extract and the pure compound.

Breeding of the high effective biocontrol strain of Streptomyces lydicus against plant fungal diseases by genome shuffling
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Phytopathology 101:S107

Streptomyces lydicus strains A01 and A02 were isolated from soil in suburbs of Beijing, China. Both of them presented strong inhibitory actions against many plant pathogenic fungi by producing nataxymycin, a polynene macroclide antibiotic with broad-spectrum antifungal activity. In this study, the 2 strains were firstly mutagenized by using complex treatment with ultraviolet ray and lithium chloride. The positive mutants E9 and E54 from A01, C16 and C23 from A02 were obtained with the nataxymycin productivities increased by 20%, 18%, 2% and 30% over that of their original strains respectively. Then the genome shuffling was carried out with the 4 positive mutants as original strains. After 2 times of recursive protoplast fusion, a recombined fusant G117 was screened out from the filial generations of the 4 parents. The nataxymycin productivity of G117 was increased by 39% over that of A01 and 56% over that of A02 respectively. Further more, the time needed for reaching the peak of nataxymycin yield for strain G117 was shortened from 72 hours to 48 hours in comparison with original strains A01 and A02. The inhibitory ratio of fermented broth of G117 against Botrytis cinerea was increased by 31%~45% over that of the original strains under the same conditions. No remarkable changes in culture characteristics, nataxymycin productivity and antifungal activity were found in strain G117 after 10 continuous generations of propagation.

The effects of temperature on the development of Amblyseius barkeri (Hughes) (Acari:Phytoseiidae)
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Phytopathology 101:S107

The development of Amblyseius barkeri (Hughes) at different constant temperatures was investigated. The results indicate that the developmental duration of A. barkeri was shortened gradually with the increasing of temperature at the temperature range of 16~28°C, and the developmental durations for egg, larva, nymph, pre-ovation and whole generation varied from 3.34d to 1.38d, 1.65d to 0.65d, 8.50d to 3.00d, 0.50d to 1.50d and 5.53d to 1.94d, respectively. However, at 30°C and 35°C, the developmental duration was prolonged for all life stages. The developmental duration of egg and pre-ovation stages were linearly correlated with temperatures, while the relationship between the developmental duration of other stages and temperatures were a parabola opening upwards. According to the priority law, the developmental threshold of pre-ovation period was 12.11°C, and all other various stages and whole generation were lower, 6.87°C (generation) and 8.03°C (larva), with an effective thermal sum of 137.58°C for generation. Based on the model of Wang Ruxong, the optimum developmental temperature for the mite was 28~30°C, critical lethal high temperature was 35.31°C~38.94°C, and critical lethal low temperature was 7.23°C~8.74°C.

Probe the interaction between SCMV PIPO with maize protein
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Phytopathology 101:S107

A cDNA library of maize (Zea mays) stems and leaves was screened with PIPO (Pretty Interesting Potyviridae ORF) cistron of SCMV-BJ to identify the inactivator by yeast two hybrid-system. Among the 51 positive candidate clones selected, two clones shared the identical sequence of 755bp containing a single open reading frame (ORF), encoding a predicted protein of 212 amino acids (a.a.) which was named PIPO. When the cloned PIPO was expressed in the transgenic maize, there was a conserved domain for the protein which was identified as maize Cytochrome P450 (YP4540). Bimolecular fluorescence complementation (BiFC) assay was used to confirm the interaction between PIPO and YP4540 in plant. The constitutively expressed fluorescence was observed in maize protoplasts and Nicotiana benthamiana leaf epidermis, respectively. The results above suggested that PIPO could indeed interact with YP4540 in living plant cells.

Metabolic profiling of xylem sap from Pierce’s disease resistant and susceptible grapevines
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Phytopathology 101:S107

Pierce’s Disease (PD) of grapevines is caused by a gram-negative, xylem-limited bacterium Xylella fastidiosa (Xf). All Vitis vinifera-based cultivars are highly susceptible to Xf infection. However, some grape species from the southern United States such as V. arizonica, V. Shuttleworthii, and Mascalumia rotundifolia are resistant. Given that Xf is limited to xylem vessels, it has been hypothesized that chemical composition of host xylem sap could play an important role in Xf pathogenesis. In this comparative study, PD resistant (9621-67) and PD susceptible (9621-94) genotypes segregating from V. arizonica × V. rupestris breeding population were used. Xylem sap samples were collected from both genotypes with and without Xf infection using a pressure chamber. The metabolic profiles of xylem sap samples were analyzed using high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS). About 20 peaks were tentatively identified as phenols, sugars, fatty acids, furfurals and furanoses by mass spectrometry. Principal component analysis (PCA) was conducted to
category compounds that are correlated with resistance/susceptibility and infected/healthy states. Metabolic profiling coupled with PCA analysis in this study can facilitate to identify and characterize genotypic differences between PD resistant and susceptible grapevines in response to β-l infection.

Fumigation and fungicide effects and quantitative and qualitative analysis of Phytophthora, Fusarium and Rhizoctonia on strawberry roots

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Phytopathology 101:S108

Black root rot (BRR), caused by Phytophthora, Fusarium and Rhizoctonia, results in significant yield reductions in strawberries. Characterization and quantification of pathogens on roots is crucial for understanding pathogen ecology and management of root diseases. Fumigation trail was set up with the following treatments: Methyl bromide/chloropirin (50:50; MC) and Picloram (PC), and fungicides using Switch (SW+); or Abound (AB+); and d applications before planting plus drip applications with Ridomil Gold after 7 days and at early spring growth and Abound at full bloom. BRR severity was lower in SW+ (5.9 Horsfall-Barret scale) and AB+ (5.3) compared to MC (6.5) and PC (6.3) and all were lower than the non-treated plots (7.0). Colony numbers of Phytophthora spp. on roots were lowest in SW+ at each sampling. Colony numbers of Fusarium and Rhizoctonia spp. composition was rich and diverse on roots within and between sampling periods. Phytophthora spp. were detected from roots before planting but not Fusarium and Rhizoctonia spp. based on culture dependent and DGGE techniques. Based on DGGE, MC and PC had the similar fungal community with AB+ suppressing fungal populations; AB dips before planting reduced Rhizoctonia. Selective use of fungicides that target known pathogens offers potential BRR management in combination or in place of fumigants when incorporated into an IPM program.

Biochemical and antibacterial properties of L-amino acid oxidase derived from Trichoderma harzianum ETS 323

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Phytopathology 101:S108

Although L-amino oxidase (LAAO) has been reported to be a potent antibacterial agent, its antibacterial mechanisms remain unclear. A novel LAAO (Th-LAAO) was isolated from extracellular proteins of Trichoderma harzianum ETS 323 and is thought to be antagonistic to the plant pathogen, Rhizoctonia solani. Here, we show that the activity and structure of this enzyme is more stable at pHs between 6 and 8 than at pH 9 or between pH 4 and 5, with the optimal pH of Th-LAAO being 7.0. Its secondary structure is estimated to comprise 35% α-helix, 17% β-sheet, 21% β-turn, and 27% random coil. Th-LAAO inhibits the growth of pathogenic Gram-negative and Gram-positive bacteria. By confocal microscopy and flow cytometry, we observed that FITC-labeled Th-LAAO inter acted with bacteria and caused positive bacteria. By confocal microscopy and flow cytometry, we observed that FITC-labeled Th-LAAO inter acted with bacteria and caused positive bacteria.

Evaluation and characterization of antifungal compounds from the fermented products of Trichoderma harzianum SL-BNR1-6

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Phytopathology 101:S108

Metabolites of Trichoderma include molecules that confer resistance to host plants (elicitors) and molecules with antimicrobial activity. Six Trichoderma strains - T. harzianum ET S323, T. harzianum ET S428, T. harzianum SLBN R16, T. virens LNPA R42, T. harzianum T22, T. virens YAM were comparatively evaluated based on the dry weight of secondary metabolites they produce by fermenting sugarcane bagasse. Trichoderma harzianum SLBN R16 was found to produce higher amount of secondary metabolites on the fourteenth day of fermentation. Further, an assay guided fractionation and purification of metabolites was performed to characterize antifungal compounds of T. harzianum SL-BNR1-6. Three anthraquinones - 2,3-dihydroxyphapacyalin, Phomarin and emodin; and a phenylpropanoid derivative - Methyl p-coumarate were identified as major antifungal compounds in the fermented products of T. harzianum SL-BNR1-6. Methyl p-coumarate and 2,3-dihydroxyphapacyalin showed higher antifungal activity against Rhizoctonia solani a well known plant pathogen. The structures were determined from respective 1H and 13C NMR spectrum.

Suppression of plant cell death and immunity by a family of Magnaporthe oryzae zinc-finger effectors

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Phytopathology 101:S108

Magnaporthe oryzae is one of the most important pathogens that devastate many cereal crops such as rice, wheat and barley. Elucidation of its virulence mechanisms at the molecular level is a prerequisite for developing novel disease control strategies through biotechnology. Bioinformatic and experimental analyses of M. oryzae genome suggest that many of the fungal proteins (so-called effectors) may enter rice cells and exploit host molecular or cellular events for the pathogen’s benefit. In this study, we have identified and characterized a 7-member family of putative effectors with a typical zinc-finger motif. Molecular and biochemical experiments indicate that this family of the fungal effectors may enter host cells in a pathogen-free manner. In addition, transient expression of these effectors in Nicotiana plants was shown to inhibit the BAX-induced programmed cell death, suggesting their potential role in suppressing plant defense response. To further investigate the role of these suppressing AB+ disease promoting effectors, we have generated stable transgenic rice lines expressing individual effectors and are currently characterizing these plants for compromised host resistance to pathogen infection. Ultimately, these efforts should lead us to a better understanding of the pathogen virulence and host immunity and facilitate the development of novel approaches for plant disease control.

Endophytic bacteria in potato tubers affected by zebra chip disease

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Phytopathology 101:S108

Potato zebra chip disease (ZCD) could drastically reduce quality and value of all market classes of potato, costing growers and processors millions of dollars in losses in North America. Endophytic bacteria colonize the internal tissue and have both positive and negative effect on their host plants. In potato, there has been research on endophytic bacteria but few were carried out in the context of ZCD. In this study, endophytic bacteria in ZCD affected potato tubers (Cultivar Atlantic), defined by the symptom of phloem necrosis and PCR detection of “Candidatus Liberibacter solanacearum”, were analyzed. A total of 85 bacterial strains belong to 17 bacterial genera were isolated from infected potato. Phylogenetic and characterized a 7-member family of putative effectors with a typical zinc-finger effectors. These results suggest that OsERF9 is integrated into the cross-talk between biotic and abiotic stress signaling networks.

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Cymene inhibition of Beauveria bassiana spor germination


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Phytophathology 101:S109

Losses due to seedling damping-off caused by Rhizoctonia and Pythium can be reduced both by seed treatment with Beauveria bassiana isolate TN11 (Bb) and by use of bioactive monarda herbades. Impact of essential oils found in monarda herbade on Bb spor germination is unknown. The purposes of this study were: to determine if cymene, an essential oil found in all monarda herbades, inhibits germination of Bb; and to develop and validate mathematical models for the impact of cymene on Bb. Six concentrations of cymene, ranging from 0.0005 to 500 µM, and a no cymene control were tested. Spore suspensions of Bb were placed on microscope slides coated with water agar, in the presence or absence of cymene-treated filters. Spore germination was observed for 24 h. After 12 h when detectable germination typically began, microscope views were photographed every 4 h, and germ tube lengths (including the diameter of the spore) were measured. Germ tube length was equal to control at concentrations at or below 0.05 µM; but germination was stimulated and germ tube length increased at 0.5 µM for all observation times and at 5 µM for most times. Spores exposed to 50 or 500 µM cymene rarely germinated. Models of spore germination tube growth rate as a function of cymene concentration were developed and model results fitted well to experimental data. Predictions of models were tested and validated by separate experiments.

Evaluation of ten leguminous cover crops as cryptic hosts for Verticillium dahliae

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Phytophathology 101:S109

Verticillium wilt is a vascular disease caused by Verticillium dahliae, challengingagricultural production of over 300 crops worldwide. The fungus produces durable microsclerotia in infected plant tissue, which can survive in soil for many years. In the absence of fumigation, suppression of soil populations of V. dahliae relies on natural attrition during periods of nonhost cultivation. The selection of legume cover crops that can function as nonhost or minimally hospitable to V. dahliae, show no symptoms of disease may nevertheless support development of the pathogen and thus negate the benefit of crop rotation. This study was undertaken to evaluate the extent to which common legume cover crops are colonized by V. dahliae and support formation of microsclerotia. Fava bean, bell bean, sunn hemp, sesbania, black-eyed peas, field pea and four vetch species were evaluated under both greenhouse and field conditions. No visual symptoms were observed and stem height of inoculated and control plants were not significantly different. However, V. dahliae was recovered from the stems of all ten crops. When infected stem segments were buried in potting mix, microsclerotia formed on decomposing tissue of eight of the ten crops. The results of this study should help to guide growers in selection of cover crops that will not aggravate problems caused by V. dahliae.

Screening strains of Trichoderma spp. for decomposition of agriculture wastes

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Phytophathology 101:S109

Trichoderma spp. are one of the important groups of fungi used commercially as biocontrol agents for the management of plant diseases. Polysaccharide hydrolyases released from Trichoderma spp. are also important source of enzymes for decomposition of agro-wastes. This study was conducted to develop a quickly technique for screening strains of Trichoderma spp. with strong capability of hydrolyzing cellulose through the activity of cellulase. Results of the experiments in solid/liquid culture media using ammonium chloride as nitrogen source showed a high degree of correlation between the index of cellulase activity/mycella growth and the activity of CMCase for the tested strains of Trichoderma spp. When the agricultural wastes, rice straw, rice bran, peanut shell, sugar bagasse or sawdust, were used as growth substrates, the enzyme activities of Trichoderma spp. varied with strains and growth substrates. On rice straw, the strain PT- MusaS24-1 released the highest amount of CMCase (119.5U), whereas the strain PTNC-WASSO-3 released the highest amount of Avicelase (29.32U) and FPA (32.05U). On peanut shell, the strain NT-TaS17-1 released the highest amount of β-glucosidase (9.53U) and on rice bran, strain PTNC-WASSO-3 released the highest amount of xylanase (176.65U). This study concludes that rice straw and rice bran are suitable substrates for production of cellulase by selected strains of Trichoderma spp., whereas saw dust is the most unsuitable substrates among the agricultural wastes tested.

New records of Tospoviruses and Geminiviruses in Mauritius

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Phytophathology 101:S109

Onion and tomato are crops of economic importance in Mauritius with annual production of 8500 and 18000 tonnes, respectively. Iris yellow spot virus (IYSV [family Bumyviridae, genus Tospovirus]), was confirmed using ELISA and RT PCR followed by cloning and sequence analysis as the causal agent of a serious outbreak of a viral disease of onion in several production areas of Mauritius. An incidence of over 80% infection was noted in onion fields in October 2009, tomato plants with reduced leaf size, leaf curling, and yellow margins associated with plant dwarfism were observed in open fields in the southern part of the island with disease incidence ranging from 5% to 50%. Tomato yellow leaf curl virus (TYLCV) (Family Geminiviridae, genus Begomovirus) was confirmed by PCR and sequencing of the amplicons. TYLCV was prevalent in open-field tomato varieties ‘Swaraksha’ and ‘Epoch’. IYSV and TYLCV are new records for Mauritius.

Exploring the Brazilian diversity of Trichoderma spp. with focus on biological control of white mold on common beans in the field


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Phytophathology 101:S109

The microbial culture collection kept by Embrapa Rice and Beans gathers over than 300 isolates of Trichoderma spp., which have been identified in the species level by means of optical microscopy and DNA barcode methods. These isolates were collected in several Brazilian regions, in order to select the best biological control agents for white mold (Sclerotinia sclerotiorum), a major disease of common beans (Phaseolus vulgaris) in the country. After dual culture, hyperparasitism and enzyme production tests carried out in partnership with the Federal University of Goias, the eight best isolates were tested in field trials in Goianira, Goias State, in 2009 and 2010. In each year, the experimental field was artificially infested with an average of 145 viable sclerotia m–2 and cropped to the indeterminate bush ‘Perola’ cultivar, under no-tillage and sprinkling irrigation. A suspension of 2 × 106 viable spore mL–1 was obtained after isolate growth at 25°C on autoclaved rice, and sprayed in the field plots during the crop V4 stage. The S. sclerotiorum inoculum density number of apothecia m–2 and disease severity were assessed, respectively, at the R5 and R7 stages. The ANOVA showed consistent results between the two trials, that the three best isolates effectively reduced the S. sclerotiorum apothecia up to 70%, with a correspondent decrease of disease severity. As a result of lower white mold severity, yields were higher in comparison to control plots and ineffective isolates.

The use of social media sites at the Plant Disease and Insect Clinic at North Carolina State University

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(1) North Carolina State University, Raleigh, NC, U.S.A.

Phytophathology 101:S109

The Plant Disease and Insect Clinic (PDIC) at NC State University provides plant disease diagnostic and insect identification services to county agents, master gardeners, producers, homeowners, and the general public. Part of effective disease diagnosis is communication and outreach to clientele. At the PDIC, we felt that we were significantly lacking in this dimension of disease diagnosis. Formerly, our website functioned primarily as a reservoir of information on sample submission instructions, fees, and links to other sites. It was not a destination page that our clientele would visit and spend time exploring. In early 2011, we developed social media pages to communicate more effectively with the public and revamped our website to be more informative, user friendly, and attractive. We began using social media (Facebook and Twitter) to broadcast current information on plant diseases and insect pests, to publicize webinars, and to alert the public to potential threats to plant life in NC. During the first two months after implementation of our social media sites, we had 963 page views on our blog, 44 followers on Twitter, and 112 monthly active users on Facebook. These sites have provided our clients with easy access to the resources and expertise of NC State University and the PDIC. Using social media sites has allowed us to reach people across the state and network with specialists, diagnosticians, and county agents from our neighboring states.
Characterization and mefenoxam sensitivity of Pythium species in North Carolina greenhouses

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Phytopathology 101:S110

Herbaceous ornamentals exhibiting symptoms of Pythium root rot were collected from greenhouses throughout North Carolina. Greenhouses were selected based on diagnostic records of Pythium-positive plants submitted through the Plant Disease and Insect Clinic (PDIC). Roots were assayed for Pythium by isolation on selective media and individual isolates were identified by morphological characterization and sequencing of the ITS rDNA region. Isolates of the predominant Pythium species were screened for mefenoxam sensitivity on 5% clarified V8 agar amended with 100 ppm mefenoxam in 48-well micro-titer plates. Isolates were evaluated using a rating scale of 0 (no growth) to 5 (completely colonizing entire well). Mean sensitivity scores 4 were considered insensitive to mefenoxam. The information obtained in this study will allow the PDIC to provide growers with control recommendations customized to their specific situation.

Seasonal fluctuation of ‘Candidatus Liberibacter asiaticus’ titers in citrus trees

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Phytopathology 101:S110

Huanglongbing (HLB) associated with ‘Ca. L. asiaticus’ (Las) is the most destructive disease of citrus. First reported in 2004, HLB has already been detected in over 300 municipalities of the states of São Paulo (SPS), Paraná, and Minas Gerais. From the Araraquara region, the initial focus of the disease, HLB moved longer distances to the south than to the north/northwest SPS, despite the widespread presence of the psyllid vector and the many citrus farms. Summer air temperatures, significantly higher in the north/northwest, may be affecting bacterium multiplication and the pattern of disease spread. In fact, in growth chambers, Las titers were 100 to ten thousand fold lower in citrus kept at daily regimen of 24–38°C than at 24-32°C (Plant Dis. 93: 257-262). In this work, 36 Las-inoculated potted sweet orange plants were maintained under screen for 32 months in Botucatu and Colina, south and north SPS. Symptom progress and bacterium titers were assessed every 2 to 3 months. Significant seasonal variation in average cycle threshold (Cts) was observed in both locations, with the highest values assessed during the summer (24.75 in Jan 2009 in Colina and 23.15 in Nov 2009 in Botucatu) and the lowest during the spring and fall (16.80 in Sept 2008 in Colina and 15.64 in Apr 2010 in Botucatu). Multiple regression analysis showed significant association between Cts and temperature. The higher the Cts the higher the number of hours above 32, 35 or 38°C registered just before sampling date.

Distribution and abundance of nematodes in corn fields in Illinois

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Phytopathology 101:S110

As a first step toward assessment of the effects of plant parasitic nematodes on corn production in Illinois, a survey was conducted to determine the distribution and population densities of plant-parasitic nematodes in corn fields in 2009 and 2010. A total of 587 soil samples were collected from 95 (of 102) counties. The survey revealed the presence of at least eleven genera, including Helicotylenchus, Heterodera, Hoplolaimus, Longidorus, Meloidogyne, Mesocricitomma, Paratylenchus, Pratylenchus, Trichodorus, Tylenchorynchus, Xiphinema, and a number of others designated “tylenchids” which were counted as a group and not yet identified to genus. The most frequently observed genera were those in the “tylenchids,” along with Helicotylenchus and Pratylenchus, which occurred in 98.8%, 95.8%, and 84.2% of the samples, respectively. The next most frequently observed genera were Heterodera, Tylenchorynchus, Paratylenchus, and Hoploaimous, found in 57.1%, 36.8%, 23.7%, and 22.0% of the samples, respectively. Population densities of each genus often exceeded estimated damage thresholds. Prominence values (PV) (PV = population density × (frequency of occurrence)/10) were calculated for each genus. Helicotylenchus had the highest PV (1634.5), followed by the tylenchids (1612.12), and Pratylenchus (286.7). The survey indicated that plant-parasitic nematodes are widespread in corn fields in Illinois. The economic importance of this information is yet to be determined.

Identifying Phytophthora species isolated from nursery irrigation water throughout North Carolina

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Phytopathology 101:S110

Species of Phytophthora are well adapted to aquatic environments, and have been commonly detected in rivers, canals, runoff water, ponds, reservoirs, streams, and hydropopic systems. The presence of pathogenic organisms in irrigation water sources can be a serious threat to plant health, especially if a grower uses reclained surface water for irrigation. During the summer and fall of 2010, irrigation water was collected from numerous ornamental plant nurseries in 13 counties of North Carolina. Water samples were assayed for Phytophthora by filtration then cultured on selective media so individual isolates could be identified by sequencing of the ITS rDNA region. Our collection of over 62 isolates included representatives of the described species P. cactorum, P. citriola, P. citrophthora, P. cryptogea, P. hydropathica, P. nicotianae, P. palmivora and P. tropicalis, as well as isolates in five clades of previously undescribed species. Future studies will include extensive monitoring at two nursery locations and sequencing of additional rDNA regions including the cytochrome oxidase II gene. The pathogenicity of undescribed species will be determined by inoculation of select ornamental hosts.

Comparative study of Phytophthora species causing carrot cavity spot in California and Michigan

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Phytopathology 101:S110

California (CA) and Michigan (MI) combined account for about two thirds of the carrot production in the U.S. One of the limiting factors of production is cavity spot, caused by Phytophthora spp. To characterize the pathogen by regions, Phytophthum spp. were isolated from carrot roots with cavity spot lesions from both CA and MI. The internal transcription spacer (ITS) of the ribosomal DNA region was sequenced for species identification. Phytophthora violae was predominant and isolated from more than 90% of all 122 CA samples; a few isolates of P. sulcatum, P. irregularare and P. ultimum were also identified in CA. In MI, four species were isolated, including Phytophthora sulcatum (38%), P. sylvaticum (32%), P. intermedium (22%) and P. irregularare (8%). In some cases, more than one Phytophthora species were isolated from one lesion. In virulence tests on carrot roots, P. sulcatum and P. violae were more aggressive than the other three species. Mycelia of P. violae and P. sulcatum grew slower than the other four species on V8 agar medium at room temperature. Mycelial growth inhibition was tested with three fungicides; all five species were sensitive to mefenoxam oxamidel and zoamide, but only P. violae was sensitive to fluopicolide. These results suggested that predominant pathogens were different between CA and MI, and that zoamide might be effective in both CA and MI, but fluopicolide is not recommended for the MI carrot industry.

Risk assessment of Phytophthora capsici resistant to fluopicolide

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Phytopathology 101:S110

Fluopicolide is a systemic fungicide affecting oomyeetes including Phytophthora capsici. A laboratory study was conducted to assess the risk of fluopicolide resistance in P. capsici. Baseline sensitivity to fluopicolide was determined using 126 P. capsici isolates from Michigan, U.S.A. All isolates were sensitive to fluopicolide and values of effective concentrations for 50% inhibition of mycelial growth (EC50) ranged from 0.0847 to 0.2380 µg/ml, with a mean of 0.1572 µg/ml in unimodal distribution. Resistant mutants were obtained at a mutation frequency above 1.0 × 10⁻² from five P. capsici isolates by screening zoospores on fluopicolide-amended (5 µg/ml) agar plates. The mutants showed either intermediate (resistance factors between 3.53 to 92.63) or high resistance (resistance factors between 2245.09 to 7034.79) to fluopicolide. The fluopicolide resistance of the mutants was stable through 10 mycelial transfers on fungicide-free medium. All resistant isolates exhibited overall level of fitness (zoospore production, cyst germination, and virulence on zucchini fruit or pepper seedlings) compared with their sensitive parental isolates, with few exceptions. Cross-resistance was detected between fluopicolide and five other fungicides, including cyazofamid, maniclidiampamid, mefenoxam, oxadixyl and azoxystrobin. Based on these results, the potential for P. capsici populations to develop resistance to fluopicolide in the field and the resistance risk may be moderately high.
Molecular mechanism of QoI resistance in Fusicladosporium carpophilum – causal pathogen of almond scab in California

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Phytopathology 101:S111

Almond leaf and fruit scab caused by Fusicladosporium carpophilum (Venturia carpophila) is a common and widespread disease in California. QoI-resistance in populations of this pathogen has been reported previously in different regions of the state. The partial cytochrome b gene was sequenced and a mutation at codon 143 (G143A) was found in all isolates highly resistant (EC50 > 30 µg/ml) to azoxystrobin. No mutations at codon 143 or 129 were found in isolates moderately resistant (EC50, 1 to 8 µg/ml) to azoxystrobin. A primer pair was designed and confirmed to target the G143A mutation. Another primer pair was designed to distinguish F. carpophilum from five common species in the genera Moniliinia, Alternaria, Botrytis, Botryosphaeria, and Aspergillus that occur on almond. Using these primer pairs, a real-time PCR assay was established to quantify the frequency of the G143A allele in F. carpophilum populations. In laboratory studies, there was a significant correlation (r²= 0.97, P < 0.001) between the proportion of spores from azoxystrobin-resistant to -sensitive isolates and the frequency of the G143A allele that was quantified by real-time PCR. This study demonstrated the potential of using real-time PCR to efficiently quantify QoI-resistance in F. carpophilum populations.

Botryosphaeria species complex associated with coast live oak (Quercus agrifolia) mortality in Southern California

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Phytopathology 101:S111

A 2009–10 survey of oak stands in San Diego and Riverside Counties was conducted to identify and characterize pathogenicity of fungi involved in oak mortality in southern California. Coast live oak (Quercus agrifolia; C. L.) mortality caused by the goldspecked oak borer (GSOB, Agrius auroguttatus) has occurred in San Diego County, California, since 2002. Diploida corticola, Botryosphaeria sarmentorum, B. hibernica, and B. stevensii were consistently recovered from bleeding CLO trunk cankers in GSOB-infested and -uninfested sites with tree mortality. Species were confirmed by ITS4/5, β-tubulin, and EF factor DNA sequencing and by morphology of ten isolates of each species on PDA-tet and on pine needle agar under UV light at 25°C, for characterizing mycelial and pycnidia characteristics, respectively. For each species, five one-year-old CLO seedlings were wound-inoculated with two isolates, using plugs from one-week old cultures, or sterile agar for controls. Every species was recovered from necrotic tissue. At three months, lesions were significantly longer than controls; however, within four weeks, D. corticola lesions extended throughout inoculated seedlings, including roots, and caused seedlings to die. Conidia occurred throughout the plant tissue, and seedlings exhibited bleeding and epicormic sprouting. Results suggest that the Botryosphaeria species complex is important in the decline of CLO at GSOB-infested and -uninfested sites throughout Southern California.

Molecular screening of walnut backcross populations for a DNA marker linked to cherry leafroll virus resistance

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Phytopathology 101:S111

Blackline disease, a graft union disorder caused by infection of English walnut (Juglans regia) trees by Cherry leafroll virus (CLRV) is a major problem for walnut production in Northern California where scions are grafted onto virus resistant black walnut (J. hindsii) or ‘Paradox’ (J. hindsii × J. regia) rootstocks. A breeding program is currently developing CLRV-resistant English walnut cultivars by recurrent backcrossing of ‘Paradox’ hybrid with English walnut cultivars. We have developed primers to detect a DNA marker specific to J. hindsii parent linked to hypersensitivity to CLRV for marker assisted selection. In PCR assays, these primers amplified a 535-bp DNA fragment from J. hindsii and ‘paradox’ rootstocks. Analysis of nucleic acid extracts from trees of a third generation backcross population by PCR indicated association of the marker with hypersensitivity to CLRV as determined by bark patch grafting inoculations. We then screened 1,174 fourth generation backcross seedlings for the presence of the DNA marker and found that 48% (563/1174) of these seedlings were positive for the marker. The molecular screening method used in this study was able to reduce time and resources that were otherwise required for screening by patch graft testing.

Evaluation of ningnanmycin for management of dollar spot and anthracnose in turfgrasses

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Phytopathology 101:S111

Ningnanmycin, a low risk bio-pesticide produced from fermentation byproducts of the soil actinomycete Streptomyces noursei var. xichangensis n. var., was evaluated for its in vitro activity against Sclerotinia homoeocarpa var. cerealis (the causal agent of anthracnose) using a three-fold dilution from 0.81 to 0.01 µg/ml. Two isolates of C. cereale and S. homoeocarpa, including three isolates sensitive to demethylation inhibitor (DMI) fungicide and three isolates resistant to DMI, were selected for the assay. Triclonazole was included as a standard for comparison. The mean EC50 values of C. cereale, S. homoeocarpa and DMI-sensitive isolates were 0.30, 0.01 and 0.17 µg/ml for ningnanmycin compared to 0.98, 0.05, and 0.49 µg/ml for triclonazole, respectively. A positive correlation was detected between EC50 values of ningnanmycin and triclonazole for isolates of S. homoeocarpa. In the field efficacy experiments, Ningnanmycin 10% WP at 0.29 g/m2 applied on a 7-day interval provided the highest control followed by Ningnanmycin 10% WP at 0.15 g/m2 (7-day interval) and Trinity 1.69S at 0.32 ml/m2 (14-day interval) for both dollar spot and anthracnose. The results of in vitro sensitivity assays and field efficacy experiments demonstrate that ningnanmycin has activities against two major turfgrass pathogens and can provide similar levels of control compared to traditional fungicides.

Screening and application of bacterial isolates as biocontrol agent against powdery mildew on cucumbers

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Phytopathology 101:S111

Powdery mildew is an important disease on cucumbers and causes a great damage in China. The control of this disease primarily depends on the application of chemical pesticides. However, it becomes a popular and important problem that many chemicals show the potential toxic effect on food, pathogen resistance and environment. So, it is a hot research topic nowadays to find an ideal product to control this disease. Microbial fungicides show such a more potential control efficacy on plant diseases in greenhouse that the studies thereof become more popular in China. In this study, 125 bacterial isolates were tested for their ability to control this disease by pot experiment and the results showed that 12 bacterial isolates significantly decreased this disease. In field plot experiment, 10 bacterium isolates of them showed a significant control efficacy. Among them, the bacterial isolate CAB-1, NZT-14-84 and BDT-25 expressed better control efficacy with 79.0%, 67.5% and 57.4% respectively. The isolate CAB-1 was identified as Bacillus subtilis. A preparation, 5 × 108 cfu/ml spores AS, was made with spores of B. subtilis CAB-1 and applied in greenhouse. Naturally infected cucumber plants were sprayed after transplanting every 7-day interval with 50-fold diluted solution of this preparation. Results showed that the treatment could significantly reduce the diseases with a control efficacy 70.5%. This study will provide a new and environment-friendly fungicide to control this disease in China.

Effects of cultural practices, Meloidogyne incognita, and Thieliavipis basica on cotton root morphology in the field

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Phytopathology 101:S111

A potential production constraint in many Arkansas cotton fields is a plow or harpod resulting from one or more of several factors may exist. Water infiltration and aeration may be restricted and soil penetration resistance increased due to impeding root penetration and exploration, and lead to suppressed plant growth and productivity. Two soilborne pathogens Meloidogyne incognita and Thieliavipis basica are also common in cotton production fields in the state. Damage to roots resulting from these pathogens may be amplified where a hardpan is also present. The objective of this study was to evaluate the topological changes in root systems resulting from these two pathogens in the presence of a competition layer. Field studies were conducted in a cotton production field in northeastern Arkansas in 2009 and 2010. Field-length strips were subsampled to a depth of 12–15 inches before planting, and strips of equal size adjacent to each subsampled strip was reserved. Telone II was applied to half of each strip prior to planting each year. Excavated root systems were analyzed from each sub-plot consistent with June and October using WinRhizo software. Nematicide application reduced galling by M. incognita but increased root magnitude, altitude, exterior pathlength and total surface area, root volume and length. Subsoiling generally increased the same
parameters numerically with less effects. Effects of both subsoiling and nematicide application were more evident early in the growing season.

Evaluation of organic sulfide fumigants for suppression of vegetable soilborne replanting diseases in greenhouse

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Phytopathology 101:S112

Soilborne-replanting diseases are seriously limiting greenhouse vegetable production in China. Authors intended to use organic sulfide fumigants to manage the diseases. Monitor on microbial populations in field soil fumigated for 5 days, 14 days, 35 ml/m², and 30 g/m², respectively showed that soil fungal density was greatly reduced by dimethyl disulfide (DADS), allyl disulfide (AD), and ethylene dimethyl disulfide (EDDS) at 30 g/m², 60 ml/m², 35 ml/m², 60 ml/m², and 35 ml/m², respectively, as determined by PDA soil plate dilution method. Sequence of toxicity of the fumigated soil bacteria was AITC > MBT ≥ DADS ≥ DMDS = STTC as by NA soil plate dilution. All the tested fumigants were highly suppressive to nematodes. Among them AITC was the most suppressive one with L0.5-0.5 µg/ml to J3 of Meloidogyne incognita. AITC and DMDS also greatly reduced weed density by 73.6-94.7% in field at 35 ml/m² and 30 g/m², respectively. Trials demonstrated that fumigation before seedling transplantation significantly decreased kidney bean Fusarium root rot and root knot diseases with control efficacy sequence AITC = MBT > DADS ≥ DMDS > STTC. Safety tests showed no injury occurred on cucumber, tomato, mask melon and cowpea when STTC, MBT, DADS, TITC, and K-Vapam were applied immediately before transplanting at less than 120, 60, 15, and 15 ml/transplanting hole, respectively. These data are instructive in formulating operation procedures of the fumigants to be used in vegetable soil treatment.

Powdery mildew biological control agents exhibit endophytic characteristics

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Phytopathology 101:S112

Two bacteria (B17A and B17B) and two fungi (F13 and F16), have been observed to exhibit endophytic characteristics by invading plant tissues. Fungal hyphae of F13 invaded the intercellular spaces of parenchyma cells, with some forming vesicular and arboreal growths in plant cells. Cells of B17B were observed under a compound microscope in the parenchyma cells, and occasionally in the vascular vessels of stained roots of young dogwood seedlings, grown in the shade house or greenhouse environments. Both B17A and F16 invaded plant cells as well, but not as frequently as either B17B or F13. The microorganisms were previously isolated from dogwoods growing in forests where no fungiceae have been used. They have also shown great potential as biological control (biocontrol) agents (BCAs) in the control of powdery mildew of flowering dogwood (Cornus florida), in greenhouse and shade house environments. Application of these BCAs individually by foliar spraying, root inoculations and root drenching have proven effective in the control of powdery mildew of dogwood, during greenhouse and shadehouse experiments. In most trials, BCAs were not significantly different from a conventional fungicide thiophenate methyl (Cleary’s 3336®), commonly used to control the disease in dogwood.

A network of field trials to test the susceptibility of rice mega-varieties to sheath blight

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Phytopathology 101:S112

Sheath blight (SB), caused by Rhizoctonia solani, is a major rice yield-reducer especially in highly intensive production systems. To date, no source of complete resistance has been identified, and no rice varieties resistant to the disease have been deployed in Asia. Mega-varieties (MVs), i.e. rice varieties grown over large acreages were tested to their susceptibility to SB. A network of field experiments was established at 5 sites for 2 successive rainy seasons from 2009 to 2010 using a common framework (inoculated line sources; split-plot design with 3 replications; disease measurements at 5 distances from the sources; four common MVs at all sites as controls; 3 observations at 10, 25, and 40 days after inoculation). Significant MV effects were consistently found, indicating that MVs differ in their susceptibility to SB. Aside from the very strong, significant distance-to-source (D) effect, a significantly D x MV was found, indicating that disease gradients, i.e., the ability of the disease to spread, differ among MVs.

Evaluation of differentiation between Magnaporthe grisea and M. oryzae by using of specific primers

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Phytopathology 101:S112

Magnaporthe grisea (Hebert) Barr is the causal agent of rice blast and gray leaf spot of grasses. Rice blast causes economically significant crop losses annually. It is now known that M. grisea consists of a cryptic species complex containing at least two biological species that have clear genetic differences. Complex members isolated from the grass genus Digitaria nomenclaturally tied to M. grisea. The remaining members of the complex isolated from Oryzae sativa and other cultivated grasses have been renamed M. oryzae. Two forma specialis names have been applied to the anamorph of M. grisea. Pyricularia grisea was described from Digitaria sanguinalis, and P. oryzae Cavara was described from O. sativa. P. oryzae was distinguished from P. grisea based on its sparse, usually nonseptate hyphae and larger, biseptate conidia. The usage of the name P. grisea and P. oryzae has generally reflected the host from which the fungus was isolated rather than any morphological differences. In this study in order to evaluation of the differentiation between these species, 26 isolates of M. oryzae and 14 isolates of M. grisea collected from north rice fields of Iran. DNA were extracted and reproduced by using m1f23 specific primers. PCR primers were derived from the sequence of the m1f23 infection–specific gene. All isolates made a 390 bp band. So, with these specific primers no genetic difference was observed between these isolates.

Grower implementation of LAMP PCR to initiate grower powder mildew fungicide program based on inoculum detection

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Phytopathology 101:S112

Inoculum detection for timing fungicide applications against grape powdery mildew is effective using qualitative and quantitative PCR (qPCR) approaches. However, implementation by viticulturist was impeded by the more than 55% accuracy in detecting 1 or 10 spores and limited isothermal PCR (LAMP) is a robust method for the detection of DNA that can be performed with minimal equipment and skill. LAMP primers were designed against the ITS2 ribosomal DNA region of Erysiphe necator that are specific and can detect less than one spore or less than 5 copies of target DNA in a purified plasmid. Continuously running an impaction spore traps where sampled every 3 months and LAMP-PCR extraction was accomplished by placing roots in 100 µl of TE buffer, centrifuging, boiling, vortexing and then placing 5 µl DNA extract in PCR tube with mastermix. The PCR tube was then placed at 65°C for 45 min followed by 80°C for 5 min. Positive detection was determined by the formation of white precipitate. Grower implementation was tested by placing 3 traps at each vineyard with one processed by the grower using LAMP and the others processed in the lab for LAMP and qPCR. Participating growers were more than 55% accurate in detecting 1 or 10 spores and over 90% accurate in detecting 100 spores in spiked samples. They had 74% agreement in detecting E. necator compared to our LAMP-PCR results, and our LAMP-PCR results were 96% in agreement with our qPCR results.

Pathogen Transport and Response-tool for Agricultural Canopys (P-TRAC) - A modeling system to guide disease management decisions in perennial canopals

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Phytopathology 101:S112

We are building a system to predict the spread of a pathogen from internal and external inoculum sources at region to sub-block scales. The system will aid in targeting disease monitoring and mitigation efforts to areas within vineyards that have a high probability for deposition and disease development.
Two years of particle dispersion data indicate that traditional dispersion modeling approaches do not adequately describe dispersion in vineyards. The Large-Eddy Simulation technique was used to model the airflow and spore dispersion and deposition in vineyards. The simulations provided high-resolution time resolved 3D distributions of momentum, heat, moisture, and spore concentration that will increase our understanding of the relationship between canopy structure, weather and disease development. The models indicated that as spores move from one row to the next, most are deflected vertically with relatively fewer deposited in or passing through the canopy. They also imply that as canopy density decreases (i.e. lower leaf area per vine and/or increased row spacing) the distance that spores travel increases but fewer spores escape the canopy. This leads to a decreased likelihood of long distance dispersion. Thus, a denser canopy would increase the focal nature of epidemics within a field but increase the potential to infect adjacent fields. In contrast, a sparser canopy will lead to an increased spread within a field but a decreased likelihood of transmission to adjacent fields.

Innate response in tissue cultured Anthurium andraeanum against Radopholus similis

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Phytopathology 101:S113

**Radopholus similis** causes Anthurium decline, a loss of vigor with smaller and fewer flowers. A disease complex occurs in Anthuriums where the plants are infected with *R. similis* and *Xanthomonas axonopodis pv. dieffenbachiae* (*Xad*) the causal agent of bacterial blight. Tissue cultured Anthuriums inoculated with nematodes did not experience significant weight loss. In a factorial design experiment, Anthurium Marian Seefurth with an average weight of 0.72 g was inoculated at the roots with 10 nematodes and 1 ml *Xad* at 10^7 CFU/ml. Plants were weighed at 1, 3, 7, 10 days and weekly for 3 weeks and monthly for 4 month. After 120 days post inoculation, anthurium roots were cut into 1 cm pieces and *R. similis* were extracted in water. Roots inoculated with *Xad* were macerated and subjected to a dilution series. Plant weight with nematode inoculation peaked at 7 days and weighed 0.6 g at 120 dpi whereas plant weight for weight with nematode inoculation peaked at 7 days and weighed 0.6 g at 120 dpi. All treatments were analyzed at 36°/30°C (16h/8h) for a week. Host range studies using several members of the Crotalaria genus showed that *Xad* co-inoculation was not able to express well at about 25°C, symptoms showing within 2–3 weeks. Symptoms in tomatoes were reported to have a wide range of pathogens in Arabidopsis

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Phytopathology 101:S113

In Arabidopsis, the R protein HRT of the Di-17 ecotype indirectly recognizes the coat protein of Turnip Crinkle Virus (TCV) and triggers R gene-mediated resistance. CRT1, an endosomal-localized ATPase, was identified in a genetic screen of mutants Compromised for Recognition of Turnip Crinkle Virus. The CRT1 gene family was shown to be required for R gene-mediated resistance against bacteria and oomycete pathogens as well as for resistance-associated cell death (Kang et al., 2008 and 2010). In addition to its involvement during several R gene-mediated interactions, we have assessed the role(s) of CRT1 family during other plant immune responses using an Arabidopsis double knock out (dkO) mutant *crtl-2 crht-1*, which lacks CRT1 and its closest homolog. Basal resistance against virulent *Pseudomonas syringae* and TCV was reduced in this dkO. Callose deposition after inoculation with *P. syringae* *avrC* mutant strain was also compromised. Moreover, CRT1 was found to interact with the PAMP recognition receptors FLS2 and EFR, as well as with their associated kinases BAK1 and BIK1. Interestingly, the dkO mutant was also defective in development of systemic acquired resistance. Furthermore, our results indicate a role for CRT1 in a set of pre-invasion defenses activated during the non-host interaction between Arabidopsis and *Phytophthora infestans*. Together, these findings argue that CRT1 is a critical component of four distinct layers of defenses and participates in defenses against a broad spectrum of pathogens.

Fusarium virguliforme genes and pathways involved in the development of sudden death syndrome in soybean

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Phytopathology 101:S113

**Fusarium virguliforme** is a soil-borne pathogen that causes sudden death syndrome (SDS) in soybean. Despite the importance of the disease, little is known about the fungal genes involved in the infection process and their expression profiles in response to plant defense mechanisms. Greenhouse assays were conducted to identify *F. virguliforme* genes expressed in planta under conditions conducive to the development of SDS. Total RNA was extracted from soybean roots challenged with *F. virguliforme* 15 days after planting. Sequencing-based transcript analysis using Illumina technology was used to identify and characterize fungal transcripts expressed in the infected soybean roots. The acquired sequences cover 20% of the publicly available genomic sequence of *F. virguliforme*. Data analysis and annotation of RNA species was performed. Subsequently, annotated fungal genes were classified into different groups based on their molecular function. The expression patterns of a subset of the identified genes were confirmed by RT-PCR. This is the first report of using next-generation sequencing to identify and characterize *F. virguliforme* genes and pathways involved in the development of SDS in soybean. These results will be used to identify new target genes to disrupt in *F. virguliforme* and study their role in SDS development.

Integration of sunn hemp cover cropping and soil solarization for reniform nematode, *Rotylenchulus reniformis*, management

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Phytopathology 101:S113

Sunn hemp (*SH*), *Crotalaria juncea*, has been known to suppress reniform nematode, *Rotylenchulus reniformis*, while enhancing free-living nematodes involved in soil nutrient cycling. Two field trials were conducted in winter 2009 (Trial I) and summer 2010 (Trial II) in Hawaii to examine if SH cover cropping could suppress reniform nematode more efficiently if integrated with soil solarization. Cover cropping of SH, soil solarization (SOL), SH followed by SAL (SOLSOL) were compared to weedy fallow (F) for 3 months prior to planting a reniform nematode susceptible host, *Vigna unguiculata*. Although SH and SOL consistently showing a trend of reducing abundances of reniform nematode, both treatments did not suppress the nematode significantly. Reniform nematodes were suppressed (P < 0.05) by SHSOL in Trial I, but not in Trial II. Thus, SOL only occasionally improves reniform nematodes suppressions and not by SH. Differences in SH biomass among treatments and trials might have caused this difference. SH biomass was higher in SHSOL than that of SH in Trial I, but a reverse was true for Trial II. Another possibility of different performance of SHSOL was that summer SOL in Trial II generated much higher heat than that of Trial I and might have reduced the allelopathic effect of SH. Thus, integration of SH and SOL does not always suppress reniform nematode more than each treatment alone, but could suppress the nematode better when the SH biomass is high and when SOL is not too hot.

A tomato model system to study Citrus huanglongbing

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Phytopathology 101:S113

Citrus greening disease (huanglongbing, HLB) has caused severe damage to citrus industries world-wide. Low tier of the HLB associated bacteria *Candidatus Liberibacter asiaticus* (LAS) in plants, prolonged latency, slow disease progression and seasonality of infection in citrus as well as lower incidence of LAS in vectors makes the citrus/HLB a challenging system to conduct research and to develop better disease management strategies. A closely related bacterium, *Candidatus Liberibacter psylla* (LPS), causes “ psyllid yellows” symptoms in tomatoes and several other annual crops. The disease is transmitted by insect, transmitted to healthy tomatoes easily with symptoms showing within 2–3 weeks. Symptoms expression in tomatoes was shown to be temperature dependent. Symptoms expressed well at about 25°C, and the bacterium titer decreased transiently when the plants were maintained at 36°/30°C (16h/8h) for a week. Host range studies using several members of Solanaceae with both graft and insect transmission of LPS revealed both resistant and susceptible plants. The suitability of the tomato/psyllid yellows model system to screen a large number of chemicals, antibiotics and transgenes for developing strategies for potential control of citrus HLB will be discussed. A BAC library constructed from infected tomato psyllids was used to generate genomic sequence information on LPS using metagenomic approach.

The CRT1 family participates in four distinct layers of immunity against a wide range of pathogens in Arabidopsis

In Arabidopsis, the R protein HRT of the Di-17 ecotype indirectly recognizes the coat protein of Turnip Crinkle Virus (TCV) and triggers R gene-mediated resistance. CRT1, an endosomal-localized ATPase, was identified in a genetic screen of mutants Compromised for Recognition of Turnip Crinkle Virus. The CRT1 gene family was shown to be required for R gene-mediated resistance against bacteria and oomycete pathogens as well as for resistance-associated cell death (Kang et al., 2008 and 2010). In addition to its involvement during several R gene-mediated interactions, we have assessed the role(s) of CRT1 family during other plant immune responses using an Arabidopsis double knock out (dkO) mutant *crtl-2 crht-1*, which lacks CRT1 and its closest homolog. Basal resistance against virulent *Pseudomonas syringae* and TCV was reduced in this dkO. Callose deposition after inoculation with *P. syringae* *avrC* mutant strain was also compromised. Moreover, CRT1 was found to interact with the PAMP recognition receptors FLS2 and EFR, as well as with their associated kinases BAK1 and BIK1. Interestingly, the dkO mutant was also defective in development of systemic acquired resistance. Furthermore, our results indicate a role for CRT1 in a set of pre-invasion defenses activated during the non-host interaction between Arabidopsis and *Phytophthora infestans*. Together, these findings argue that CRT1 is a critical component of four distinct layers of defenses and participates in defenses against a broad spectrum of pathogens.
Managing pest risk of plants for planting in international trade: U.S. import regulations at a crossroad

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Phytopathology 101:S114

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The Animal and Plant Health Inspection Service (APHIS) has proposed a comprehensive revision of U.S. import regulations for plants for planting, contained in the Code of Federal Regulations, Title 7, Part 319, Subpart 7 (CFR 319.7). The proposal of the regulatory revision has been reviewed with three major rules, which are in different stages of development. First, a new category of regulated plants will be established, whose importation is not authorized pending pest risk analysis (NAPPPRA). Pest plants and plant hosts of quarantine pests meeting criteria established by APHIS will be included in the NAPPPRA category. The second rule will establish a system of controlled import. APHIS will revise the Departmental Permit system to reflect current practices and appropriate pest risk management. The third rule will establish a framework for the structural reorganization and consolidation of regulations affecting imported plants for planting, and integrated measures approaches for pest risk management. These measures are being developed to reduce pest risk while minimizing adverse economic impacts on international trade in plants for planting. The U.S. is a net importer of live plants, with increasing trends in trade from all world regions.

The potency of fungal antagonists to combat root rot in industrial Acacia mangium plantation

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The root rot disease had caused plant death in Acacia mangium with increasing number of dead plants over time and rotation. The pathogen remains in the field within the infected plant roots and stumps, as well as rotting logs of the infected tumbled trees. Three types of different root rot pathogens had been identified at some root rot spots in South Sumatera Indonesia, and the antagonistic agents isolated from the area were tested separately against the three root rot pathogens using double culture method. From the 45 samples collected from the root rot damaged area, mostly in form of the pathogen fruit bodies, with few wood-skin and root cuts, 18 fungal isolates were gained, and 14 of them had antagonistic potency of 90–100% at day three towards the three most commonly found root rot pathogens, which were identified as: Ganoderma lucidum, Ganoderma aureae, and Rigidosporus microporus. The antagonistic fungi were mainly identified as Trichoderma spp. and Gliocladium spp. Further tests showed that representatives of the Trichoderma spp. and Gliocladium spp. isolates were able to grow and sporulate on the Acacia mangium leaf litters to yield spores with similarly high antagonism potency to the different root rot pathogen types tested, whether applied singly or as a mixture of the two types. Therefore there is good possibility for the antagonistic fungi to be able to combat root rot in large industrial plantation such as in Acacia mangium.

Seasonal distribution of SI fungicide resistance in apple scab populations in Virginia

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Apple scab, caused by Venturia inaequalis, is an economically devastating disease that occurs wherever apples are grown. Sterol inhibitor (SI) fungicides have been dominant systemic fungicides used to manage scab. Unfortunately, V. inaequalis is developing resistance to the Sls. We evaluated fungicide resistance in 266 single-spored V. inaequalis isolates collected in Winchester, VA from 2006 to 2010. Within a given season, the mean colony growth was significantly different (P < 0.001) among assay treatments (0.1, 0.5 or 1.0 ppm myclobutanil) and assay times (7, 14, 21 or 28 days). Sampling interval was significant (P < 0.001) in 2007 and 2008, and pairwise comparisons suggested variations between early and late season. When analyzed concurrently, all factors were significant (P < 0.001) including collection year. Percent growth suppression (PGS) – the difference in colony growth on 0 and 1 ppm myclobutanil at 28 days – was used to assess fungicide resistance. Generally, a range of resistance was seen at each sampling interval, and the average PGS was similar for treated and non-treated trees of the same cultivar. The average PGS in the July (30%) sampling interval was lower (i.e. more resistant) than in the May (60%), June (60%) or August (50%) sampling intervals. Of the total 266 isolates evaluated, approximately 8% were classified as resistant, 29% as moderately resistant and 63% as sensitive. This 5-year study provides useful information about the seasonal distribution of SI fungicide resistance in VA's apple scab population.

The USDA-APHIS quarantine programs for sugarcane, grasses, rice & bamboo

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Phytopathology 101:S114

Since its transfer to Plant Protection and Quarantine (PPQ) of the USDA-APHIS in October, 2005, the Plant Germplasm Quarantine Program (PGQP) has received an increasing number of introductions and species belonging to the Poaceae family, which are imported primarily as vegetative propagative materials and occasionally as seed. These importations include sugarcane, turf and forage grasses, Miscanthus, bamboo and rice. Since October 2005, PGQP has entered more than 325 sugarcane clones into quarantine, 51 of which were clones that re-entered the program after coming out of our virus elimination program in addition, PGQP has established approximately 57 bamboo accessions, 304 Miscanthus and 24 Arundo donax introductions for biofuel evaluations, 153 turf and forage grasses, and 726 viable rice accessions. Each clonal introduction is subjected to an array of tests which include: bioassays; leaf dip assay via electron microscopy; isolation and culture; and serological and PCR-based tests to detect a specific pathogen or the pathogen group that includes grass pathogens. Germplasm that consistently tests negative for pathogens of quarantine interest and appears healthy during growth is released to the original requestor. Infected germplasm may be subjected to virus elimination via apical meristem culture. Pathogens detected from imported Poaceae germplasm will be presented.

A functional 3-hydroxy-2-butanone pathway is required for virulence in Pectobacterium carotovorum subsp. carotovorum

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Phytopathology 101:S114

The Pectobacterium are ubiquitous soft rot pathogens (SRP) responsible for wilting, necrosis and massive maceration symptoms in potato, vegetables and ornamental plants. Like other Enterobacteriaceae plant pathogens, such as Dickeya, Enterobacter and Erwinia, Pectobacterium species encode genes involved in the production of acetoin (3-hydroxy-2-butanone, 2HB). 2HB is a volatile metabolite produced via the Embden-Meyerhof pathway. Previously we found that an operon encoding 2HB pathway enzymes, budAB, was highly expressed during P. carotovorum subsp. carotovorum (Pcc)-potato stem and tuber interactions. We found that a Pcc mutant with a deletion of budR, which encodes alpha-acetolactate synthase, is significantly reduced in its ability to macerate potato tubers compared to the wild-type strain. The mutant was not impaired in bacterial growth in tuber tissue during infection, which suggests that this pathway is not required for nutrient acquisition. Additionally, this mutant also inhibits alkalinization of growth medium and tuber tissue under anaerobic conditions. An increase of pH in the plant and bacterial cell creates the activity of pectinases that are involved in plant cell wall degradation. Thus, this data suggests that the acetoin pathway in SRP may contribute to pathogenesis through pH modulation. Finally, although 2HB has been reported to promote plant growth, we were unable to demonstrate any effect of these compounds on potato plantlets grown in tissue culture.

Assembly of the draft genome of Xanthomonas axonopodis pv. dieffenbachiae strain V108LRLUH1, a bioleuinscent strain highly virulent on anthurium

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Phytopathology 101:S114

A severe limitation to the large scale production of anthurium is bacterial blight caused by Xanthomonas axonopodis pv. dieffenbachiae (Xad). Although some cultivars of anthurium have exhibited tolerance to the disease, high levels of resistance or immunity are not commonly observed in Anthurium andreanum cultivars. To gain insight into the underlying plant-microbe interaction, we have used Roche 454 sequencing technology to sequence the genome of Xad strain V108LRLUH1, a well-characterized bioleuinscent (Lux*) strain. This sequencing process has generated 582,110 36 bp reads. The de novo assembled genome sequence of Xad V108LRLUH1 reads has yielded 215 contigs involved in plant cell wall degradation. The genome sequence of the strain indicated that the acetoin pathway in Xad is greater than 5Mbp in size with a GC content of approximately 65%, similar to other members of the Xanthomonas genus. Genes associated with virulence (i.e. effectors, secretion system, etc.) will be compared to those of other Xanthomonas strains. This is the first X. axonopodis pv. dieffenbachiae strain to be sequenced and will represent the first sequenced genetically modified xanthomom. The genome content of Xad and further phylogenic comparisons of this strain to other
Xanthomonas genomes will provide a better understanding of bacterial evolution and plant-microbe interactions.

A new broad-spectrum fungicide for use on lentil, field pea, and chickpea crops

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Phytopathology 101:S115

BAS 703 01 F is a new broad-spectrum fungicide developed in Canada by BASF Canada for broad-spectrum control of key fungal diseases of lentils, field peas, and chickpeas. BAS 703 01 F is a premix fungicide containing two active ingredients, fluxapyroxad and pyraclostrobin in a 1:1 ratio. Fluxapyroxad is a new fungicide developed by BASF which inhibits respiration of fungi by blocking production of succinate dehydrogenase and will be classified in FRAC group 7. Field efficacy trials have indicated that BAS 703 01 F is highly effective at controlling diseases such as Ascochyta blight (Ascochyta lentis) and anthracnose (Colletotrichum truncatum) of lentil; Mycosphaerella blight (Mycosphaerella pinodes) of field pea; and Ascochyta blight (Ascochyta rabiei) of chickpea in the rate range of 150 – 200 g/ha.

Field trial results from 2009 and 2010 and proposed directions for use will be presented. BAS 703 01 F has been submitted to the PMRA for registration.

Blueberry necrotic ring blotch, a new blueberry disease caused by a virus

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Phytopathology 101:S115

Novel symptoms have been observed on southern highbush blueberries (Vaccinium corymbosum interspecific hybrids) in several southeastern states. Affected plants show irregularly shaped circular spots or blotches with green centers on the top and bottoms of leaves. Diagnostic tests failed to isolate any fungal or bacterial pathogens typically associated with such symptoms. Double-stranded RNA (dsRNA) was extracted from symptomatic leaves suggesting the presence of virus(es) possibly involved in the disease. Three of five dsRNA segments observed on gels have been sequenced and used to develop diagnostic primers for detection by RT-PCR. More than 50 individual plants that exhibited necrotic ring blotch symptoms in North Carolina, Georgia and Florida were positive in the RT-PCR assay that was developed. The perfect correlation between the virus and symptoms in plants from across several states suggests that the virus this, for which we propose the name Blueberry necrotic ring blotch virus (BNRBV), is indeed the causal agent of the disease. Sequence analysis showed that BNRBV has conserved replicase and movement domain characteristics of other ssRNA viruses. Because no coat protein conserved domains have been identified, high throughput sequencing is being used on dsRNA preparations to determine if one of the other common dsRNA virus coat protein code for a coat protein, or if additional virus(es) may be involved in the disease. BNRBV is related most closely to Citrus leprosis virus.

Identification of an emergent bacterial blight of garlic in Brazil

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Phytopathology 101:S115

Outbreaks of a bacterial blight disease occurred on garlic (Allium sativum) cultivars Roque Caxiense, Quiteria and Cacador in Southern Brazil, and threatened the main production regions of Rio Grande do Sul State. Symptoms were characterized by water-soaked reddish streaks along the leaf midrib, followed by yellowing of leaves, a rot of bulbs and plant death. The disease can negatively impact seed production in infested fields. Epidemics occurred mainly during bulb formation and the preharvest period. Bacteria, fluorescent on King’s B medium, were isolated from leaf tissue. Physiological tests indicated that the bacteria were Pseudomonas marginalis. Pathogenicity tests were performed on plantlets and detached cloves. Symptoms similar to those in the field were reproduced on the leaves. Additionally the bacteria incited a rot of clove. Pathogens had physiological properties identical to the inoculated strains, demonstrating Koch’s postulates. DNA fragment pattern analysis using DNA amplified with the BOXA1R primer, demonstrated that two different pathogens were responsible for the disease. The DNA fragment patterns were different than the type strain of P. marginalis and the pathotype strains P. marginalis pv. pastinaceae, P. marginalis pv. alfalfa. Although the strains appear to be similar to P. marginalis according to physiological tests, further research is needed to determine if the bacteria represent a novel pathovar or species.

Colonization of spinach (Spinacia oleracea L.) by GFP-tagged Verticillium dahliae

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Phytopathology 101:S115

The soilborne fungus, Verticillium dahliae, causes wilt in a wide range of hosts, including spinach (Spinacia oleracea L.). The interaction between a conserved replicase protein (GFP)-tagged V. dahliae strain and spinach was studied by confocal laser scanning microscopy. The roots of spinach seedlings were inoculated with a conidial suspension of a GFP-tagged strain and pathogen colonization events were followed through seed production. At 24 to 96 hours post-inoculation (PI), conidia germinated and formed hyphal colonies on root tips and in the root elongation zones. Two weeks PI, hyphal growth in the root cap and intercellularly in the cortical tissues and penetrated into the xylem. At six to eight weeks PI, the fungus colonized the entire taproot xylem with abundant mycelia and conidia. Further colonization of the taproot and crown of inoculated plants led to vascular discoloration when foliar symptoms became apparent. At 10 weeks PI, xylem tissues of the upper stem were colonized that also extended to the inflorescence and the various spinach seed parts, including fruit wall, epicotyl meristem and integument. However, the fungus did not colonize the pepsiperm (the diploid maternal tissue) in the seed. This information is useful in administering effective seed treatments without compromising seed viability and ultimately the introduction of this very destructive pathogen via seed.

Egg parasitoids of Chrysocoris javanus Westw. (Hemiptera: Scutelleridae) on Jatropha curcas L. in Bogor, West Java, Indonesia

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Physic nut (Jatropha curcas) is one of biofuel plants which is planned to be cultivated on large scale areas in Indonesia. Chrysocoris javanus is an important sucking pest attacking physic nut. To control this pest, various pest control measures have to be studies and implemented. The objectives of this research were to find out egg parasitoids which are potential as biological control agents and their parasitization level at three physic nut plantations in Bogor, West Java, Indonesia. Egg parasitoids found during the study were Anatusp. sp. (Eupelmidae), Perlomalidae, and Scelionidae (Hymenoptera). Parasitized eggs of C. javanus were black in color, whereas the unparasitized eggs were orange. The permealid was the dominant parasitoid found in two plantation. Parasitization level of three parasitoids ranged from 60.1% to 97.0%. Almost all of C. javanus egg clusters were parasitized (88.7% - 100%).

Relative efficacy of chemical management tools on Phytophthora crown and root rot of pepper plants

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The oomycete pathogen Phytophthora capsici can cause extensive losses in pepper plantings. Fungiecides are an important component of this Phytophthora disease management system. Several products have recently been registered and some additional new chemistries are being developed with activity against oomycete pathogens. Two greenhouse trials were conducted in 2010 to evaluate these individual products for their ability to suppress development of crown and root rot on pepper plants in the presence of P. capsici. Within a series of 600 ml capacity plastic pots, a 2-month-old chile pepper transplant was placed into either a peat-based potting mix (first trial) or a silty clay loam field soil (second trial), both infested with vermiculite containing P. capsici. The potting mix or soil in each of the 10 replicate pots per treatment was drenched with one of the test products at the initiation of each experiment and again 14, 28 and 42 days later. Plants were watered daily for the 2-month duration of each trial. No untreated plants were alive at the conclusion of the trials. In contrast, the mean survival rate for treated chile pepper plants in both experiments was 85% for Ridomil Gold (mefenoxam), 80% for Revus (mandipropamid), 65% for Forum (dimethomorph), 60% for Omega (fluazinam) or V-10208, 55% for Presidio (fluopicolide), 45% for Zampro (manetocardin + dimethomorph), and 25% for Ranman (cyazofamid).

Reevaluation of Phomopsis species affecting sunflowers in the United States

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Phytopathology 101:S115
Phomopsis Stem Canker (PSC) is a serious sunflower disease in Europe, but has remained at a low incidence in the United States until recently. In 2010, PSC affected 8.7% of the U.S. crop, and yield reductions occurred in isolated fields. Phomopsis helianthi was thought to be the sole infectant of PSC in Europe, but some researchers hypothesized more species were involved. PSC infected sunflower stalks were collected from infected fields in Minnesota (MN), North Dakota (ND) and South Dakota (SD) in 2010. Identification of Phomopsis isolates using morphology and molecular analysis of the internal transcribed spacer region (ITS 1, 5.8S ribosomal RNA gene, and ITS 2) revealed four Phomopsis/Diaporthe species – namely Diaporthe helianthi, Diaporthe stewartii, Phomopsis longicolla and an unknown Diaporthe sp. Our research indicates that multiple species are associated with PSC of sunflowers in United States.

Screening of the World Phytophthora Collection for viruses

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Phytopathology 101:S116

The presence of viruses in fungi and oomycetes, including a few Phytophthora spp. is well established. These viruses can affect host phenotypes including pathogenicity, which has led to their use as biocontrol agents. The World Phytophthora Collection (WPC) is maintained on the UC Riverside campus in the Department of Plant Pathology and Microbiology and includes over 10,000 accessions from multiple species and geographic locations, types of incidence and species of mycoviruses present in this collection. 200 accessions were selected for screening by double-stranded (ds) RNA analysis which can detect the presence of both double-stranded and single-stranded RNA viruses. DsRNAs have been detected in over a dozen isolates with several unique patterns, some indicating the possible presence of mixed viral infections. A large dsRNA segment (approx. 13KB) was found in five isolates and is believed to be an endornavirus that has been previously associated with Phytophthora spp. RT-PCR of the dsRNAs is being conducted to confirm this association. Several of the dsRNA positive cultures grow very slowly and the relationship of this phenotype to the presence of a mycovirus is being investigated. Virus particle isolations are underway as is continued screening of the WPC for dsRNAs. This wide ranging survey of Phytophthora isolates could result in the eventual development of a viral biocontrol agent for important plant diseases.

Understanding cellular and molecular interactions between the rice blast fungus and a putative biocontrol bacterium

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Phytopathology 101:S116

Lysobacter enzymogenes is a gram negative, soil-dwelling bacterium that interacts with many microbes and lower eukaryotes. This bacterium also produces lytic enzymes and antibiotics, and by virtue of its antagonism, has great potential as a biological control agent. The details of L. enzymogenes' interactions with other organisms are not well-understood. In order to better understand them, we used a model plant pathogenic fungus, Magnaporthe oryzae. Our goal was to investigate this interaction using molecular and cellular tools. We generated a fluorescent dsRed-C3 (wildtype strain) of L. enzymogenes and a Zs-green strain of M. oryzae. Live cell imaging using confocal microscopy was used to examine the fungal-bacterial interactions. M. oryzae strain 70-15 was grown on oatmeal medium for 10 days. Six ml of L. enzymogenes (1 × 10^6 CFU/ml) in phosphate-buffer saline solution was added to the plate. The bacterial-fungal interaction was imaged for 24 hours. Our results indicated that the wild type bacterium readily and quickly attaches to fungal hyphae and spores, and between 3–4 hours, many more attachments are seen. At approximately 12 hours, bacteria cover 70–80% of the fungal material. No internalization was observed of bacteria into live fungal cells. mRNASeq of fungal gene expression during early and late stages of the interaction is currently underway and will be discussed.

Detection and population dynamic analysis of biological control agent Pseudomonas fluorescens LB3W1 in tomato plants from the ‘Live coating seed’

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Phytopathology 101:S116

The ‘Live coating seed’ is the newly developed pelletized seeds, in which useful microorganisms are coated alive using a combination of decompression and dehumidification technology. This technique improves the efficacy of biological control agents (BCAs) for plant diseases. In the present study, detection and periodical movement of a BCA, Pseudomonas fluorescens LB3W1 (3W1), in the tomato plants from the live coating seed, were investigated using serological and molecular biological methods. The antiserum against 3W1 used in this study was raised in a rabbit by immunization with heat treated antigen. The characteristic of the serum was tested by indirect ELISA. As a result, the titer value and specificity of anti-3W1 serum were suitable for bacterial detection. Furthermore, the immunofluorescence method using FITC conjugate was also admitted to be useful applicable, in particular for qualitative movement analysis on specific sites of the plant. The specific PCR primer targeted 3W1’s gvdB gene sequence was designed and tested for the specificity and detection limit. The bacterial populations in plant parts were examined by these methods. The results obtained from different methods were strongly correlated, suggesting the applicability of these methods for population dynamic analysis of BCA on plant from ‘Live coating seed’.

Cultural control of maize wallaby-ear symptom: Damage avoidance by early planting of forage maize

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Recent global warming has brought many pests and diseases from tropical to temperate region. Maize wallaby ear symptom (MWES), which had occurred only in tropical areas, newly occurred in temperate Asian countries such as China and Japan after 1980s. In Southern part of Japan, this symptom now occurred on maize and other crops. To overcome this problem we investigated using serological and molecular biological methods. The etiology and control of this disease was investigated. Pathogenicity was evaluated on parsley, celery (Apium graveolens), and coriander (Coriandrum sativum). Buffer (0.01 M phosphate, pH 7.0) or suspensions of bacteria in buffer (at approximately 10^8 CFU/ml) were inoculated by spraying until runoff. All plants inoculated with bacteria developed leaf spots. DNA fragment banding patterns of the fluorescent bacteria in parsley and degrees of MWES occurrence is depending on density of the leafhopper and plant growth stage (leaf stage); MWES occur seriously on young maize (less than 5 leaf stage) attacked by more C. bipunctata. These facts suggest that planting of forage maize in earlier season before C. bipunctata density increases in field is effective to avoid MWES occurrence. We verified this hypothesis by field experiments. In our census field, density of C. bipunctata remains low from spring to early July, then, it rapidly increases in late July and reaches maximum in September or October. Non or less MWES was observed when forage maize was planted before late July, however, MWES occurred on maize planted after early August, and the degrees of MWES become serious depending on delay of planting date. These results indicated that earlier planting of forage maize can avoid MWES occurrence in Southern part of Japan.

A new disease of parsley (Petroselinum crispum) in California caused by a fluorescent pseudomonad related to Pseudomonas viridiflava

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In 2008 fluorescent bacteria were isolated from bacterial leaf spot symptoms on Italian parsley (Petroselinum crispum) in Ceres, California. These isolates were different from the known bacterial pathogens of parsley in California. To determine the etiology of the disease, pathogenicity was evaluated on parsley, celery (Apium graveolens), and coriander (Coriandrum sativum). Buffer (0.01 M phosphate, pH 7.0) or suspensions of bacteria in buffer (at approximately 10^8 CFU/ml) were inoculated by spraying until runoff. All plants inoculated with bacteria developed leaf spots. DNA fragment banding patterns of the original eight isolates and the fluorescent reisolates from symptomatic tissue were identical to each other by BOX-PCR and differed from Pseudomonas syringae pv. apti, P. syringae pv. coriandricola and P. viridiflava. BLAST was used to compare the 16S rDNA gene sequences from the parsley isolates to those in public databases. The 16S rDNA sequences from the parsley isolates were identical to the 16S rDNA sequence of the type strain of Pseudomonas viridiflava. Although rpOD and gvdB sequences of the parsley isolates were most similar to those of P. viridiflava, they were not identical. These results indicated that an unknown pathogen isolated from parsley was related to but not identical to P. viridiflava. Further taxonomic work is needed to determine if these isolates are variants of P. viridiflava or represent a new pathovar or species.
Rhizoctonia solani AG-8 and Pythium ultimum are important soil-borne fungal pathogens of wheat that cause annual yield reductions of 5-30% in Washington State. Rhizobacteria in raw soil and to test a subset of strains for colonization in raw soil. PCR-based screening indicated that several strains carried genes for production of extracellular polysaccharides, exoprotease, and cyclic lipopeptides. The differences in motility and production of extracellular polysaccharides, exoprotease, and cyclic lipopeptides. PCR-based screening indicated that several strains carried genes for the production of the antifungal metabolites 2,4-DAPG, PCA, pyrrolnitrin and pyoluteorin. Colonization assays revealed that nine strains were capable of maintaining populations of log 5 CFU g−1 root throughout four cycles of wheat plantings. Most strains were capable of controlling R. solani AG-8, but P. ultimum was inhibited by only three strains. The most promising strain, 15D11, was a persistent colonizer capable of controlling both pathogens in planta. This strain has potential for development of a consistently-performing biopesticide for control of root diseases of wheat.

Complete genomes of plant growth-promoting rhizobacteria Pseudomonas fluorescens strains Q8r1-96 and Q2-87.

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We report here complete sequences of genomes of P. fluorescens strains Q8r1-96 and Q2-87, two plant growth-promoting rhizobacteria that originate from a take-all decline soil in Washington state, U.S.A. The genome sequences were obtained using a combination of Sanger and 454 pyrosequencing technologies. The genome of Q8r1-96 is comprised of a 61% GC circular chromosome of 6.6 Mbp and encodes 5,783 proteins, 61 tRNAs, and 9 rRNA operons. The 6.4-Mbp genome of Q2-87 has GC content of 60.7% and encodes 5,689 proteins, 62 tRNAs, and 10 rRNA operons. Both strains are plankmid-free but carry a number of mobile genetic elements. Despite the fact that Q8r1-96 and Q2-87 are very closely related, their genomes share only 82% of shared protein-coding content. Both strains carry a number of environmental fitness and biocontrol determinants including type III and type VI protein secretion systems and genes for production of 2,4-diacetylphloroglucinol, hydrogen cyanide, CLPs, antimicrobials, and putative enterotoxins. Q8r1-96 and Q2-87 also carry putative rhizosphere colonization determinants and traits that favor plant growth. Among these are the ability to produce gluconic acid and solubilize inorganic phosphate, and to utilize plant-derived phenolics, gamma-aminobutyric acid, and volatiles aceton and 2,3-butanediol. Q8r1-96 also carries a gene for 1-aminocyclopropane-1-carboxylic acid deaminase and can stimulate root growth by lowering plant ethylene levels.

Characterization of a rare Plum pox virus W isolate found in germlasm illegally carried to the U.S.

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Plum pox virus (PPV) was identified in GF-305 seedlings bud-grafted with three Prunus accesses from Ukraine that were illegally hand-carried to the U.S. without official permit. One of the accesses was typed as PPV D and the other two as PPV W, a rare strain described on a single plum tree in Ontario, Canada in 2003. Serological characterization using sub-specific antibody detection indicated that the 44189 isolate does not belong to PPV D, M, Elam or Cherry subgroup. Several monoclonal antibodies (MAb), specific to PPV W isolate 3174 from Canada, were tested. PPV isolate 44189 from Ukraine tested negative with PPV W MAb 10G7 and 2C3 and positive with 4C11 and 6B6 MAb in triple antibody ELISA and/or Western Blot. Coat protein sequence comparison of W3174 and W44189 isolates revealed amino acid deletions and substitutions in MAb 10G7 and 2C3 epitope sites. Full-length sequencing of the isolate 44189 genome is 85% complete. RT-PCR forward primers were designed and combined with previously described reverse primer (James and Varga, 2004, Acta Hortic. 657:177-182) for identification of PPV W. Our experience with characterization of the 44189 isolate at the amino acid and nucleic acid level highlights the need for constant improvement and re-validation of existing molecular and serological tools to achieve accurate detection and identification of new and unusual PPV isolates, especially from new geographical areas.

Active manipulation of resident biotype to suppress Macrophomina phaseolina in strawberry

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Phytopathology 101:S117

M. phaseolina is a pathogen of emerging importance in strawberry production systems. Brassicaceae seed meal amendments suppressed proliferation of M. phaseolina through soil systems, but optimal seed meal-induced pathogen suppression required a functional soil biology. Suppression of M. phaseolina was obtained with seed meal sourced from various brassicaceae species and was not associated with production of a biologically active chemistry (e.g. allyl isothiocyanate by Brassica juncea). Seed meal-induced disease control was temperature sensitive and suppression of M. phaseolina root infection attained at 28°C was abolished when assay temperature was elevated to 32°C. Wheat cultivation alone or in conjunction with B. juncea seed meal application was highly effective in suppressing M. phaseolina root infection when strawberry was planted into a naturally infested field soil. Interestingly, treatments that suppressed or abolished strawberry root infection by M. phaseolina did not consistently suppress quantity of the pathogen detected in bulk soil. Disease control was associated with an overall increase in density of fungi recovered from rhizosphere soil.

Advances in Brassicaceae seed meal formulation and application for replant disease control in organic apple orchards

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Phytopathology 101:S117

Brassicaceae seed meals when used independently do not provide uniform and sufficient control of the pathogen complex that incites apple replant disease. Trials were established at multiple sites to evaluate the efficacy of seed meal formulations for control of this disease in organic production systems. When amendments were applied approximately one month prior to planting and tarped with a virtually impermeable film, a Brassica juncea/Sinapis alba seed meal formulation significantly improved apple tree growth and suppressed the target pathogen complex at two (STM and Tukey) of the three orchard sites. At the third site (SR), seed meal amendment resulted in significant phytotoxicity and approximately 40% tree death. Application of the seed meal formulation in the autumn prior to planting at SR orchard resulted in tree growth that was equivalent to that attained in response to pre-plant soil fumigation. The seed meal formulation reduced in-row weed coverage by approximately 85% at the STM orchard and weed suppression was evident at the end of the growing season. These preliminary data indicate that the seed meal formulation may be as or more effective than Telone-C17 fumigation for control of replant disease, but that plant back periods and seasonal application requirements will vary with soil type.

Woody host plant problems in Maryland diagnostic clinics from 2008–2010

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Phytopathology 101:S117

The University of Maryland Plant Diagnostic Laboratory processes ca. 800 samples per year. Diagnoses are determined by microscopy, isolation, ELISA, Biolog, and Agdia virus testing. Woody hosts made up 37% of samples submitted to the diagnostic lab from 2008–2010. Diagnoses from this period were 29% fungal and oomycete, 25% abiotic, 11% insect-related, 5% bacterial, 2% unknown/insufficient sample, and 0.4% nematode/virus. Additionally, 27% of diagnoses were “no pathogen found,” indicating that no specific abiotic factor or infectious disease agent could be identified. Common diagnoses included environmental stress, Phytophthora root/crown rot, Botryosphaeria bark canker scabs, Swiss needlecast (Phaeocryptopus gaeumannii), scales/borers, and bacterial leaf scorch. Homeowner inquiries are received via email at a separate facility, the Home and Garden Information Center (HGIC). Because no physical samples are submitted, conclusive diagnoses are not possible at this location. From 2008–2010, 2,767 woody host questions were submitted to the HGIC. Of those, 67% dealt with plant problem issues (18.5% insect, 14.3% disease, 34.2% abiotic); the remaining 33% were on weed control and plant selection/culture. The lower amount of disease reported with 4C11 and 6B6MAbs in triple antibody ELISA and/or Western Blot.

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Phytopathology 101:S117

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Plant Diagnostic Lab and the HGIC address diagnoses with different methods, they work jointly to answer plant problem questions for all Maryland citizens.

Using metconazole as a seed treatment to protect sugarbeets from early season Rhizoctonia Crown and Root Rot

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Phytopathology 101:S118

Rhizoctonia solani Kuhn is found in the soil of all sugar beet growing regions. It is the causal pathogen of Rhizoctonia Crown and Root Rot (RCCR), an economically important disease of sugarbeets. Recent reports have noted the increase in the distribution and severity of RCCR. In extreme situations, RCCR can destroy up to half of the crop. Currently there are no registered seed treatments to reduce root rot. Metconazole has been found to provide protection against invading foreign DNA. The content of each CRISPR array can differ in both repeat number and in the presence or absence of specific spacers. Seventy-two strains of Erwinia amylovora (Ea) vary in geographic isolation, host range, plasmid content, and streptomycin sensitivity were evaluated for CRISPR array number and spacer variability. The CRISPR repeat sequence among Ea strains consists of 29 bp and is universal despite deletions in Ea1189. MetaCRISPR is a systemic, triazole fungicide that has shown activity against R. solani in other crops. It was applied at a rate of 0.2 grams of active ingredient per 100,000 seeds, on pelleted blank seed or incorporated into the pelleting. Metconazole has been shown to provide a reduction in infection severity of RCCR and early season survivability in the greenhouse. In the field, metconazole has increased stands and reduced infection percentage from RCCR. Metconazole, applied as a seed treatment, has been shown to provide protection from early season RCCR.

Erwinia amylovora CRISPR arrays provide an effective tool for evaluating species diversity and microbial source tracking

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Phytopathology 101:S118

Clustered regularly interspaced short palindromic repeats (CRISPRs) comprise a family of short DNA repeat sequences found in approximately half of all bacterial and archaeal genomes. These repeats are separated by non-repetitive spacer sequences that, in combination with a suite of Cas (CRISPR-associated) proteins, are thought to function as an adaptive immune system against invading foreign DNA. The content of each CRISPR array can differ in both repeat number and in the presence or absence of specific spacers. Seventy-two strains of Erwinia amylovora (Ea) vary in geographic isolation, host range, plasmid content, and streptomycin sensitivity were evaluated for CRISPR array number and spacer variability. The CRISPR repeat sequence among Ea strains consists of 29 bp and is universal despite host range or other variables. A total of 536 unique spacers were identified in the 3 CRISPR arrays present in Ea. CRISPR arrays 1, 2, and 3 could be subcategorized into 20, 16, and 2 pattern types, respectively. Spacer patterns from Michigan strains were mainly distinct from strains isolated in the western U.S. although strains from Europe and the Middle East shared the same patterns as some strains from Michigan. Host of isolation was also a factor in spacer diversity, with Rubus and Indian Hawthorn Ea isolate spacers distinct from those Ea isolated from pome fruit. Spacer homology was present among both clan I and II strains. Sixteen spacers segregated for the trait and two putative major quantitative trait loci (QTLs) affecting spacer homology were identified. These findings potentially provide the first genetic solution to the control of take-all. The identification of wheat cultivars that reduce take-all inoculum build-up could help to significantly reduce yield losses in consecutive wheat crops.

Fungicidal sensitivity of Podosphaera xanthii and efficacy of fungicides with resistance risk for cucurbit powdery mildew in New York in 2010

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A leaf-disc assay was used to determine sensitivity of the cucurbit powdery mildew (FM) fungus to currently registered fungicides in research and commercial plantings. After the last fungicide application in the research plantings, the highest frequency of strains resistant to boscalid (tolerant of 500 ppm) was in plots where Pristine (boscalid and pyraclostrobin; FRAC Code 7) and Procure (triflumizole; Code 3) were applied alone every week (70% where Pristine was applied at lowest label rate and 29% where applied at highest rate, compared to 10% where an integrated program was used) and the highest frequency of strains tolerating 10 ppm quinoxyfen (20%) was in Quinoxyfen (Code 10) plots (compared to 0% where an integrated program was used). Quinoxyfen was very effective while efficacy of Pristine and Procure (triflumizole; Code 3) declined during the season resulting in ineffective control for the entire season based on AUDPC for severity on lower leaf surfaces. The bioassay conducted in pumpkin crops mid-season on 31 Aug revealed that 0%–24% (12% avg) and 40%–100% (61% avg) of the pathogen populations were resistant to boscalid and code 11 fungicides, respectively, while 0%–1% and 0%–7% tolerated 10 ppm boscalid and 2 ppm myclobutanil. The bioassay on 21 Sept the end of the PM management period revealed that 11%–70% (40% avg) of the pathogen population was boscalid-resistant and 0%–2% tolerated 10 ppm quinoxyfen. Degree of control in these fields reflected frequency of boscalid resistance.

Molecular identification of Galactomyces species and population structure of the two postharvest sour rot pathogens of fruit crops in California

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Sour rot caused by Galactomyces citri-aurentii (Gca) and G. geotrichum (Gg) is a major postharvest decay of fruit crops in California (CA). Species-specific rDNA has been used to determine the presence of the endo-polygalacturonase and beta-tubulin genes differentiated isolates of the two morphologically similar species. Isolates were collected from agricultural soils and decayed fruit from CA and worldwide locations, and were used in population genetic studies based on AFLP markers. Four geographical sub-populations (3 CA counties and locations outside CA) among 97 isolates of Gca and two sub-populations (within or outside CA) among 35 isolates of Gg were defined. For both species, the proportion of polymorphic loci and haplotypic diversity were high. Indices of genetic differences (FST) among sub-populations within each species were all low (0.0384 to 0.2263) indicating a low level of genetic differentiation. The effective migration rate Ne was calculated as 1.709 to 2.862 for Gca and 12.53 for Gg migrations per generation. Following clone correction, mating type segregation ratios for Gca did not significantly (P > 0.1) deviate from a 1:1 ratio for all four sub-populations, indicating a random mating structure. Tests of the index of association IA and parsimony tree-length permutation tests also supported a random mating structure for both species. A mixed reproduction system with an out-crossing sexual mating system and a prolific asexual phase is proposed for both species.

Reducing take-all inoculum build-up during a first wheat crop

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Phytopathology 101:S118

Globally, take-all (Gaeumannomyces graminis var. triticii) is regarded as the most important root disease of wheat and when severe can be devastating to wheat productivity. The occurrence of severe take-all depends on the amount of inoculum surviving in the soil when a susceptible wheat crop is sown. The effect of wheat cultivar on take-all inoculum build-up (TAB) in the soil during a first wheat crop has been investigated in field experiments over multiple years. After harvest a soil core bioassay method is used to gauge the amount of take-all inoculum left in the soil. A cross-season REML variance analysis revealed that there are consistent differences between wheat cultivars in their ability to build-up take-all inoculum in the soil during a first wheat crop (9 cultivars tested over four growing seasons, P < 0.01). This trait has also been identified in a wider range of current commercial UK wheat cultivars in a 2009 field experiment (45 cultivars, P < 0.001). The genetic basis of the TAB trait is being explored in an Avalon (A) x Cadenz (C) double haploid (DH) mapping population. The A x C DH mapping population was shown to segregate for the trait and two putative major quantitative trait loci (QTLs) have been identified. These findings potentially provide the first genetic solution to the control of take-all. The identification of wheat cultivars that reduce take-all inoculum build-up could help to significantly reduce yield losses in consecutive wheat crops.

Characterization of novel genes involved in Erwinia amylovora pathogenesis

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Phytopathology 101:S118

The enteric pathogen Erwinia amylovora is the causative agent of fire blight. Mutual analyses of the E. amylovora genome have yielded important insights into the genetic determinants required for full virulence by E. amylovora. To date key pathogenicity factors include the exopolysaccharide amylovoran and the hypersensitive response and pathogenicity (hrp) type III secretion system (T3SS). Here we report the characterization of three novel genes involved in E. amylovora pathogenesis. Microarray analyses identified three novel genes (nipl, ydcN and EAM_2938) that are regulated by HrpL, a master regulator of T3SS. YdcN is a predicted XRE transcriptional regulator that may regulate genes important in E. amylovora pathogenesis. Nipl is a lipoprotein implicated in the secretion of extracellular DNA (eDNA), encoding a novel role for eDNA in plant-microbe interactions. EAM_2938 encodes a putative membrane protein of unknown function. Chromosomal deletions in ydcN, ydcN and EAM_2938 genes were generated and also subjected to phenotypic analyses. E1189Δnipl, E1189ΔydcN and E1189ΔEAM_2938 were all required for full virulence in immature pear; while E1189ΔydcN and E1189ΔydcN also exhibited alterations in swelling motility and biofilm formation. In addition, two genes, hrpF and hrrQ encode putative components of the T3SS. Both E1189ΔhrpF and E1189ΔhrrQ...
were non-pathogenic when inoculated into immature pears, highlighting the important role of type III secretion.

Evaluation of commercial algaeicides to mitigate Phytophthora spp. in naturally-infested water

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Phytopathology 101:S119

Few management options exist to mitigate Phytophthora species in water. Because Oomycetes are closely related to brown algae, application of commercial algaeicides to infested water may prevent the dissemination of propagules of Phytophthora spp. We evaluated the efficacies of four commercial algaeicides (three copper-based products and one hydrogen dioxide product) to Phytophthora spp. in naturally-infested water from two streams in western South Carolina. In each month of 2010, replicate aliquots of water (15 liters) from each stream were placed in 19-liter containers that remained in the stream to maintain ambient temperature. Algaeicides were applied at the maximum label rate. Each month, one algaeicide was tested at lower rates to identify minimum efficacy levels. Before and at 2 and 4 hours after treatment, 200-ml subsamples of water were filtered through polycvinylidine membranes (5 µm pores) and filters were inverted onto a selective medium to allow colonies of Phytophthora spp. to develop. Phytophthora spp. were detected each month in both streams in non-treated water. However, Phytophthora spp. were not detected in water after treatment with the three copper algaeicides but were detected on occasion in water treated with the hydrogen dioxide product. Copper-based algaeicides appear to be effective throughout the year, over a range of temperatures, and at several rates; therefore, they may prove to be an effective management strategy for Phytophthora spp. in some water systems.

Transmission of the opportunistic cotton (Gossypium hirsutum L.) boil pathogen Pantoea agglomerans by the brown stink bug (Eusichistus servus Say)

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Phytopathology 101:S119

Damage to developing cotton bolls by piercing-sucking insects such as stink bugs has traditionally been attributed solely to pest feeding. Previously, we showed clear differences in severity of boil damage resulting from southern green stink bug (Nezara viridula L.) fed sterile food compared to those fed food contaminated with a rifampicin (Rif) resistant, opportunistic Pantoea agglomerans strain (Sc 1-R). Insects not exposed to Sc 1-R caused localized wounding at the feeding site, whereas seed and lint necrosis occurred in bolls pierced by bugs infected with Sc 1-R. Eusichistus servus (Say), the brown stink bug (BSB), is another key pest of cotton. Here, we examined whether adult BSB could vector Sc 1-R. Sterilized green beans were dipped in either sterile Ho or a suspension of Sc 1-R. Next, BSB were provided either of the food sources (2-d), and then sterile beans (5-d). BSB were then caged with a greenhouse boll at 2 weeks post-anthesis (5-d). Bolls were examined 2 weeks later. No disease was evident in bolls with wounds caused by control BSB; yet bacteria were detected from respective seed and lint tissue on non-selective media (10^4 cfu/g) and no growth on Rif amended media. Disease was observed on an AV2 open reading frame, the presence of an N-terminal PWRLMAGT motif in the capsid protein and phylogenetic analyses indicated that ToLCPEV was more similar to DNA-A components of bipartite New World begomoviruses than to monopartite viruses from the Old World. Mutational analyses revealed that ToLCPEV-DNA-A ac4 mutants were infectious in tomato and Nicotiana benthamiana plants but did not induce symptoms, whereas av1 (capsid protein) and ac1 (Replication-associated protein) mutants were not infectious. Thus ToLCPEV is one of the first examples of a bona fide monopartite begomovirus from the New World.

Detection, diversity, and molecular characterization of closteroviruses infecting Hawaiian ti (Cordyline fruticosa L.)

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Phytopathology 101:S119

The ti plant (Cordyline fruticosa L.) is culturally important throughout most of Polynesia and has considerable economic importance in Hawaii where the foliage is commonly used in cultural ceremonies as well as ornamental and food industries. Ringspot symptoms were recently observed on leaves of the common green variety of ti, dramatically reducing commercial production of cut foliage. Sequencing of high-molecular-weight double-stranded RNAs isolated from symptomatic tissues revealed the presence of four distinct closteroviruses, which were designated Cordyline virus 1 (CoV-1), CoV-2, CoV-3, and CoV-4. Phylogenetic analyses using the Heat Shock Protein 70 homolog could not assign any of these viruses to current genera within the family Closteroviridae. A reverse-transcription PCR assay was developed for the detection and discrimination of these viruses. Based on this assay, it appears unlikely that any of these viruses are the causal agent of ti ringspot, as they could be found in both symptomatic and asymptomatic plants. It was also found that individual ti plants often harbor multiple viruses, and the geographic distribution of these viruses in Hawaii is not uniform, suggesting vector transmission of these viruses. This is also supported by the detection of these viruses in ornamental ti varieties recently derived from seed. Further molecular characterization of these viruses and identification of a vector will contribute to our knowledge on the diversity of the family Closteroviridae.

Evolutionary ecology of invasion in the omics era: Examining inbreeding depression and invasion success of the common horsetail, Solanum carolinense

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Phytopathology 101:S119

Solanum carolinense is a perennial, invasive weed found in cropfields and pastures. The ability of some families to self-fertilize may aid the invasion of new environments. If individuals with contrasting breeding histories vary in their ability to respond to ecological stresses, it may reduce the evolutionary advantages of selfing potential. The project presented here focuses on the use of comparative metabolomics and functional genomics to analyze the differential expression of genes triggered by herbivore damage between plants with contrasting breeding history (selfed vs. outcrossed). Individual ramets belonging to five families that vary in selfing ability were assigned to each of two treatments: herbivore damage (damaged or undamaged) and breeding history (outcrossed or selfed). The herbivory treatment involved a 48-h feeding trial using Manduca sexta caterpillars. Total RNA was extracted from a commercially available agent. In a greenhouse, radish and tomato were grown in potting soil infected with S. stelliscabiei (10^5 CFU/cm^2). Radish biomass was...
significantly ($P < 0.05$) higher (337% increase of fresh roots and 365% increase of fresh leaves) in pots treated with Bacillus spp. BAC03 (10^6 CFU/cm^3) than the non-treated. Potato growth was promoted (157% fresh weight increase) compared to non-treated controls. In order to identify the mechanisms associated with the antagonistic activity, non-ribosomal peptide synthetase were extracted with acid precipitation and ribosomally synthesized proteins were precipitated with ammonium sulfate, and both were tested against Streptomyces spp. isolates. A protein was derived in responding to antagonistic activity. Further identification and characterization of the compounds are ongoing.

Evaluation of systemic acquired resistance inducers for control of basil downy mildew

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Phytopathology 101:S120

Basil downy mildew (BDM), caused by Peronospora belbahrii, is a devastating foliar disease recently discovered in South Florida in 2007. BDM has spread to over 20 states and has become a major threat to sweet basil production in the U.S. Current management strategies including the use of fungicides are inadequate. In this study, five systemic acquired resistance (SAR) inducers i.e., Actigard® (ASM), 3-amino-butanolic acid (BABA), isonicotinic acid (INA), salicylic acid (SA), and sodium salicylate (NaSA) were evaluated in the greenhouse for their potential to control BDM. Foliar sprays of ASM applied as pre- (P), post- (PO) or pre+ post (PP) inoculations at rates 25 to 400 mg/L significantly ($P < 0.05$) reduced BDM severity from 35.4 to 3.5% compared to the non-treated control (CK). Foliar spray of ASM at 50 mg/L 3 days after inoculation (DAI) resulted in a 93.8% reduction in BDM severity, but only a 48.9% reduction was achieved when applied 7 DAI. BABA sprayed at rates up to 100 mg/L failed to control BDM. However, BABA applied as PP at high rates showed a significantly improved efficacy against BDM from 81.1 to 91.8%. In vitro tests indicated that ASM and BABA at rates lower than 1.0 mM had no effect on sporangial germination of P. belbahrii. INA, SA and NaSA evaluated at various rates and timings varied in the effect on BDM. ASM or BABA at low rates followed by foliar sprays of Prophyt® and Quadris® significantly reduced BDM severity compared to either of the SAR inducers alone.

Effects of green manures on nematode population densities in an organic tomato field

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An increased demand for organic foods has resulted in a greater need for pest management strategies in organic vegetable production systems. To this end, cover crops have been implemented as green manures in organic tomato field to be studied for effects on nematode population densities. Treatments were: 1) mixed species hay (Festuca arundinacea, tall fescue; Dactylis glomerata, orchard grass; Phleum pretense, timothy; Trifolium pretense, red clover; Medicago sativa, alfalfa); 2) Vicia villosa (hairy vetch); 3) F. villosa plus Secale cereale (rye); 4) V. villosa plus Raphanus sativus (forage radish); and 5) a bare soil control, six plots/treatment. Cover crops were incorporated with a chisel plow in April 2010. Plots were sampled before incorporation, ~2 weeks later, midseason and harvest. Meloidogyne was found in only 3 plots, at the second sampling time. Total plant-parasite nematode numbers/100 cc soil at that time were 21.3, 2.5, 33.8, 26.3, and 8.3 (means for treatments 1-5, respectively), and at harvest were 26.9, 40.8, 46.7, 43.8, and 40.8. No significant differences were found among treatments or dates, and no root galling was observed. Marketable tomato yield means ranged from 2.6 lbs (or two selected plants/plot) for hairy vetch+rye to 5.9 pounds for hairy vetch; no significant differences were found among treatments. The results indicate that under low nematode pressure, the cover crops did not result in any change in nematode populations.

Mating disruption for Planocephalus fuscus S.: How to successfully initiate a novel sustainable control tool

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Phytopathology 101:S120

Mating disruption has become an important tool in Integrated Pest Management programs, particularly for lepidopteran pests. Over a three year field trial we have investigated mating disruption for another pest, the vine mealybug, Planocephalus fuscus (Signoret) (Hemiptera, Pseudococcidae). The vine mealybug infests some vineyards in Mendoza, Argentina, and is one of the more important vineyard mealybugs in the world. This insect excretes honeydew, which promotes sooty molds, and vectors grape leafroll associated viruses (GLRaV). The possibility to use mating disruption for vine mealybug presents challenges but offers the potential ecological wine grape production in the affected areas. In field trials, a synthetic sex pheromone was released in plastic dispensers at a rate of 600 dispensers/ha. Trials were conducted at two mealybug infestation densities (Low: High), and with or without pesticide applications. Results showed that at high infestation densities the pesticides must also be applied to control the mealybug in the first year, but a reduction was seen in the second year also without pesticides. In vineyards with low mealybug infestation densities, effective control was provided in the first year. Results also showed an initial reduction of the number of vines affected, and after the second year a reduction in the number of fruit clusters damaged. A recommendation is made to apply mating disruption for a minimum of three seasons to provide effective control.

Genetic structure and patogenicity of Phytophthora infestans sensu lato collected from Solanum betaceum in southwestern Colombia

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Phytopathology 101:S120

Late blight caused by Phytophthora infestans is a limiting and devastating disease in several solanaceous crops in the tropics. In South America, specific P. infestans populations have been characterized, associated with exotic tropical fruits. These populations are able to infect different wild and cultivated hosts causing important economic losses. In this study, Phytophthora infestans sensu lato isolates collected from Solanum betaceum in Nariño and Putumayo states were analyzed using phenotypic and genetic features. Aggressiveness tests were realized using a detached leaf bioassay. Isolates belonged to the A1 mating type. la mtDNA haplotype and EC-3 chemotype. Results obtained with microsatellites markers (SSRs) showed that the population structure varied from clonal populations previously reported in Colombia, suggesting high levels of genetic diversity among all isolates. Our results also revealed high levels of variation in aggressiveness among the isolates when tested on susceptible and resistant cultivars. In detached leaf bioassays we observed that isolates did not infect Solanum tuberosum, suggesting host specificity. These results have significant implications for the understanding and characterization of the evolutionary history and epidemiology of the pathogen in the North Andean highlands.

Molecular and phenotypic variation of German populations of Fusarium graminearum causing head blight in wheat

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Phytopathology 101:S120

Fusarium graminearum sensu stricto (s.s.) causing head blight (FHB) in wheat is a destructive pathogen. In a survey of 12 populations with each of 30 heads from naturally infected wheat fields in Germany, F. graminearum s.s. dominated the Fusarium population with 64.9% out of 521 single-spore isolates. All three chemotypes were identified by PCR assays with a dominance of 15-acetylenoviolanal enolome (92%). The twelve populations showed high allelic diversity. PCR based gene coding for 1-3 hectares in 300 haplotypes out of 338 isolates revealed by 19 microsatellite primers. Genetic diversity within populations (72%) was considerably higher than among populations (28%) as shown by analysis of molecular variance (AMOVA). Three of the populations were additionally analyzed phenotypically for mean FHB rating and deoxynivalenol (DON) content on a moderately susceptible wheat genotype in two locations and two years. F. graminearum s.s. were revealed significantly ($P < 0.01$) genotypic variation within each population for both traits. Partitioning of genotypic variance revealed similar values like AMOVA. In conclusion, F. graminearum s.s. populations in Germany displayed a tremendous genetic variation on a local scale. Multiple resistance genes of different origin should be introgressed in breeding programs to obtain a long-term stable FHB resistance.

Antifungal compounds in ripe fruit from a resistant blueberry cultivar suppress infection by Colletotrichum acutatum

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Phytopathology 101:S120

Anthracnose fruit rot, caused by Colletotrichum acutatum, is among the most important diseases of blueberries. Most cultivars are susceptible but ‘Elliott’ is resistant. Our objective was to identify possible antifungal compounds that play a role in this resistant response. Initially, chemical fractions from lyophilized ripe fruit of ‘Elliott’ and the susceptible cultivar ‘Jersey’ were

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extracted with water, methanol, and ethyl acetate. Extracts were screened on solid media for suppression of microconidiation of *C. acutatum*. The methanolic extract was further fractionated and the soluble methanolic fraction from 'Elliott' was the most biologically active. This fraction was dried, dissolved in water, and screened in vivo by pre-treating ripe 'Jersey' fruit using 0.5, 1, 2, and 4% solutions and subsequently inoculating the fruit with *C. acutatum*. An 88% reduction in infection incidence was observed after 12 days with the 4% solution. Anthocyanins and flavonols were then quantified in fruit of the two cultivars using HPLC-MS. 'Elliott' fruit contained more anthocyanins (5.38 mg/g of freeze-dried tissue) than 'Jersey' (3.75 mg/g of freeze-dried tissue); however, the same compounds were found in both cultivars. Additionally, several unique flavonols were present in 'Elliott' (four distinct peaks). Further purification and identification of these compounds will provide new insights into the role of anti-fungal compounds in the resistance response in 'Elliott' fruit.

The SA and ET signaling pathways mediate tomato resistance to bacterial wilt at cool temperatures

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Phytopathology 101:S121

Salicylic acid (SA) and ethylene (ET) signaling pathways play an important role in tomato defense responses against bacterial wilt disease caused by *Ralstonia solanacearum*. Tomato plants infected with cool-tolerant Race 3 biovar 2 strain UW551 upregulated genes in both the ET and SA defense pathways. The speed and amount of defense gene induction predicted the degree of host resistance. Interestingly, temperature significantly affected host defense gene expression. Defense genes were more strongly expressed at 20°C than at 28°C in tomato plants with comparable pathogen populations. We measured wilt disease progress at 20°C and 28°C after transgenic salicylic acid-degrading NahG and ethylene-insensitive Never Ripe tomato plants were infected with strain UW551. Never Ripe was more susceptible to bacterial wilt than its wild-type parent, confirming the importance of ET for disease resistance. This effect was more pronounced at 20°C than at 28°C. Disease progress in NahG and wild-type tomatoes was comparable at 28°C, contrasting with the gene expression results that implied SA involvement in host resistance. At 20°C however, the NahG-transgenic line was significantly more susceptible to *R. solanacearum* than its wild-type parent. Collectively, these data suggest that the relevant tomato defense responses are inhibited at elevated temperatures, leading to increased susceptibility to infection by *R. solanacearum*.

Phenotyping Yr17 resistance in wheat to stripe rust and Yr17 virulence in *Puccinia striiformis* var. *tritici* E. Milus (1), K. LEE (1)
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Phytopathology 101:S121

Yr17 has been used as an all-stage resistance gene to protect wheat from stripe rust caused by *Puccinia striiformis f. sp. tritici*. However, it has been difficult to accurately determine Yr17 resistance in the field. Multiplex PCR reactions for simultaneous detection of multiple races for race identification. The objective of this study was to determine the effects of post-inoculation temperature on the host-pathogen interaction. Seedlings of 21 wheat lines with Yr17 (based on a linked molecular marker) were inoculated with two avirulent races (PST-3, PST-78) and two virulent races (AR10-04, PST-127) and inoculated at 10°C, 18°C, and a gradually-changing regime from 10°C-18°C. Disease progress was recorded 20 dai using the 0–9 scale for stripe rust in which 0–4 is avirulent/resistant and 5–9 is virulent/susceptible. The two virulent races were similar and were incorrectly identified as avirulent 13, 12 and 36% of the time at 10, 5–18 and 18°C, respectively. PST-3 was incorrectly identified as virulent 10, 5 and 0% of the time at 10, 5–18 and 18°C, respectively. PST-78 was incorrectly identified as virulent 68, 44 and 6% of the time at 10, 5–18 and 18°C, respectively. A set of primer was most effective at 18°C, but none of the temperatures promoted accurate phenotyping of wheat lines or *P. striiformis* isolates. It may be more accurate to designate Yr17 as an adult-plant resistance gene. PST-78 may be heterozygous for virulence on Yr17.

Comparison of old and new strains of *Puccinia striiformis* f. sp. *tritici* for ability to initiate stripe rust epidemics in wheat E. Milus (1), D. MOON (1)
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Phytopathology 101:S121

Since 2000, a new strain (based on AFLP phenotype) of *Puccinia striiformis* f. sp. tritici has replaced the old strain in eastern United States, and stripe rust has been more severe than before 2000. Epidemics begin from overwintering infections that develop into discrete foci by spring. The objective of this research was to determine if the strains differed for ability to initiate epidemics from overwintering infections. Plants of a susceptible cultivar were grown outdoors, inoculated lightly with isolates AR90-01 and AR97-01 (representative of old strain) and AR00-05 and AR03-33 (representative of new strain), inoculated in a dew chamber, and transplanted into field plots of the same cultivar at Fayetteville and Kibler, AR, during the falls of 2007 and 2008. Random pots were inoculated in a growth chamber to determine the levels of initial infection for each isolate, and levels among isolates were either similar or higher for isolates of the old strain. To quantify the amount of disease in the spring, the average severity across all stems in 0.5-m lengths of the two rows adjacent to each transplant was determined, and data were analyzed using analysis of variance. Isolates of the new strain consistently caused significantly more stripe rust than isolates of the old strain, indicating that isolates of the new strain are more aggressive than isolates of the old strain for initiating epidemics, and this helps explain why stripe rust has been more severe since 2000.

Evaluating artificial microRNAs for engineering resistance against tospoviruses

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Phytopathology 101:S121

MicroRNAs (miRNAs) are a highly conserved class of small non–coding RNAs, which are both highly specific and effective to achieve silencing of genes. We are using artificial microRNAs (amiRNA) technology for introducing resistance to Tomato spotted wilt virus (TSWV). amiRNAs were developed targeting viral RNA sequences encoding the nucleocapsid protein (N) and the silencing suppressor (NSs) genes of TSWV. An Arabidopsis thaliana miR159 precursor was modified to express virus-specific amiRNAs. Transient expression of amiRNAs in *Nicotiana benthamiana* by agroinfiltration has confirmed expression of virus-specific amiRNAs by Northern blot analysis. The ability of the amiRNA constructs to confer resistance to TSWV has been confirmed in virus challenge experiments. Stable Arabidopsis and tobacco plants have been generated with selected constructs. We are investigating various construct design features to improve the efficiency of expression of the mature amiRNAs that would provide effective resistance against tospoviruses.

Application of multiplex PCR to mixed populations of tomato bacterial pathogens

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Phytopathology 101:S121

Diagnosis of foliar bacterial diseases of tomato has been enhanced with polymerase chain reaction (PCR) and primers for the detection of pathogens. Furthermore, specific primers have been developed to detect specific pathogens in mixed bacterial populations. Previous work in other laboratories has focused on multiplex PCR reactions for simultaneous detection of multiple species of bacterial pathogens using pure cultures. The objective of this study was to optimize a multiplex PCR protocol for specific detection of *Xanthomonas* species, *Pseudomonas syringae* pv. *tomato*, and *Clavibacter michiganensis* subsp. *michiganensis* in field samples of tomato foliage. The primers selected were RST 65 and RST 69 (*Xanthomonas* spp.), MM5 and MM7 (*P. syringae* pv. *lycopersici*), and MM5 and MM7 (*C. michiganensis* subsp. *michiganensis*). Temperature parameters and concentrations of reaction components for single PCR reactions with these pathogens and their respective primers were harmonized to allow for DNA amplification of the three pathogens in one reaction. The resulting multiplex PCR gave optimal results for all three primer pairs at an annealing temperature of 57.2°C. Pure cultures were used to develop the protocol. The sensitivity and specificity of the assay was discussed. The multiplex PCR protocol for evaluation of field samples will allow for rapid identification of these pathogens, facilitate population studies, and provide a valuable diagnostic tool.

Morphological and Molecular diagnosis of *Corynespora cassicola* and *Cercospora* sp. causal agents for hydrangea leaf spot diseases

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Phytopathology 101:S121

Hydrangea leaf spot disease often referred to as *Cercospora* leaf spot was associated with six pathogenic fungi with *C. cassicola* and *Cercospora* sp. having the highest frequency of occurrence as the major pathogens. The two fungi, *C. cassicola* and *Cercospora* sp. do not produce spores readily in culture and their morphological identification can be challenging. While *C. cassicola* cause small discrete lesions less than 10 mm in diameter and larger marginal non-discrete lesions, the lesion similarity to those caused by
Cercospora complicate disease diagnosis. In addition, variability of symptoms caused by C. cassicola in different hydrangea cultivars was observed to farther complicate the identification of C. cassicola as the primary pathogen. Misdiagnosis of C. cassicola may inflate the severity of Cercospora in hydrangea leaf spot diseases. To aide identification of C. cassicola and Cercospora sp., morphological distinction between the two fungi in culture were evaluated, differential cultivar reactions to C. cassicola and Cercospora sp., were identified and specific primers were developed as molecular diagnostic tools. DNA sequences for C. hydrangea has not previously been deposited at the GenBank, and the DNA sequence of Cercospora sp. pathogen of hydrangea matched to that of C. beticola, C. fukashiana and, C. penzigii.

Association of Plum pox virus M strain with plum fruit dropping in Iran

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Phytopathology 101:S122

Severe fruit dropping was recently observed in some plum orchards of Mazandaran province; in order to investigate possible involvement of Plum pox virus (PPV), 75 leaf and shoot samples collected during the summer late and autumn of 2010. Sampling was based on typical PPV symptoms. Serological diagnosis was made by DAS-ELISA using a commercial PPV polyclonal anti- serum (Bioreba). Molecular detection was made by trapping virus particle with the above polyclonal antisera and IC-RT-PCR was performed by using the general pair of primers P1/P2. Total RNA were after enzyme digestion showed all of PCR products contained the target¬ing (Cter) CP, using P1/PD and P1/PM pair of primers that were confirmed the presence of the PPV CP gene. The results of RT-PCR analysis were in complete agreement with the DAS-ELISA and IC-RT-PCR results. The type of strain determined by RT-PCR target¬ing (Cter) CP, using P1/PD and P1/PM pair of primers that were identified and specific primers were developed as molecular diagnostic tools. DNA sequences for C. hydrangea has not previously been deposited at the GenBank, and the DNA sequence of Cercospora sp. pathogen of hydrangea matched to that of C. beticola, C. fukashiana and, C. penzigii.

Field resistance of selected banana cultivars against Tropical Race 4 of Fusarium oxysporum f. sp. cubense in the Philippines

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Phytopathology 101:S122

Recent epidemics of Fusarium wilt caused by virulent Tropical Race 4 of Fusarium oxysporum f. sp. cubense (Foc) posed serious threat to the Philippine banana industry. Based on monoculture cropping of TR-4 susceptible Cavendish varieties, the nation’s multi-million US$ export banana industry is at risk. To develop disease management tactics, selected introduced and local cultivars were studied for their reaction to Foc in a field previously severely affected by TR4. Evaluated were two commercially grown Cavendish, and 2 variants from Taiwan, 3 local cultivars and 1 improved hybrid from Honduras. The experimental plot comprised of 10 tissue-culture derived plants spaced 2.5 × 3 m, replicated 10 times, and arranged in completely randomized block design. Disease incidence was assessed weekly by monitoring any symptom including leaf chlorosis, wilting and/or pseudostem splitting. The 2 commercially grown Cavendish, Grand Naine and Williams, showed susceptible reactions, with incidence of more than 90% before shooting. Lakatan, a popular local cultivar was most susceptible with 100% incidence. GC/TCV119, and Formosana, Cavendish variants did not show any symptoms in the first crop, although symptoms were appearing in the ratoon crop. Sabah, an important cooking banana was highly resistant with no disease incidence, even in the ratoon crop. Vegetative Compatibility Group (VCG) analyses confirmed that Foc TR4 VCG1213/16 was associated with the infections.

Agrobacterium-mediated transformation of sugarcane with the anti- apoptotic gene CED-9 confers abiotic stress tolerance

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Phytopathology 101:S122

Embryogenic calli of sugarcane (Saccharum officinarum L.) genotypes TCP87-3388 and CP72-1210 were transformed with the anti-apoptotic gene CED-9, a C. elegans homolog of the mammalian Bel-2 cytoprotective gene family. Transformed plants were selected on culture medium containing Genetecin, and 5-iodo-2′-deoxyuridine (5-IddUrd) for mini- and microcalli. The transgenic lines were evaluated for drought tolerance at two different developmental stages; 40 and 90 days post germination, with water deprivation periods of 10 and 20 days, respectively. Candidate drought tolerant plants were recovered in both tests. The selected dehydration water periods represent the minimum amount of time after which wild type plants were unable to recover (even after rehydration). Selected transgenic lines remained viable and were not impaired in development. These results suggest that the anti-apoptotic gene CED-9 integrated into the genome of sugarcane may confer drought tolerance. Further experiments are underway to investigate the role of CED-9 in other abiotic stresses, including salinity, cold, and d heat.

Development of an in vitro multiplication method of sugarcane transgenic lines to improve stress tolerance screening

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Phytopathology 101:S122
Because of the high number of transgenic sugarcane lines needed for the efficient screening of candidate plants tolerant to abiotic stress, the development of a robust propagation system is required. With traditional vegetative propagation methods, each sugarcane plant under greenhouse condition will be able to generate 6 to 10 T2 plants in a 6–8 month period. In order to improve this rate-limiting step, a protocol for in vitro propagation of transgenic lines was developed. Plants were regenerated via organogenesis from the meristematic region of the leaf rolls. Preliminary results show that using this novel method, a 10 fold increase in the number of plants generated was achieved compared to traditional methods (6–10 plants with traditional method versus ~60 plants with in vitro propagation). Additionally, using this method, we were able to use younger plants (4 months in greenhouse after the tissue culture process) compared to the mature plants (~8 months in greenhouse after the tissue culture process), thus significantly reducing the propagation timeline. Taken together, this method increases the number of propagated plants, reduces the propagation time by half, substantially decreases the amount of space needed, and dramatically increases the number of repetitions per treatment. Transgenic lines propagated by this method are being used in preliminary in vitro screens for abiotic stress tolerance, such as heat, cold, salt and drought.

Application of a real-time PCR assay for detection of eastern filbert blight in hazelnut breeding

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Phytopathology 101:S123
Eastern filbert blight (EFB) is a devastating disease of European hazelnut, Corylus avellana L., which causes economic losses in Oregon, where 99% of the U.S. crop is produced. The incitant, Anisogramma anomala (Peck) E. Müller, is native east of the Rocky Mountains, where it is harbored by the American hazelnut (C. americana Marshall). While C. americana is tolerant, EFB causes dieback and death of C. avellana. Detection and identification of A. anomala in nursery stock and breeding populations is challenging and time-consuming using conventional methods, because disease symptoms show only after 16 months from infection, and the fungus can only be cultured from infected material. Transgenic lines propagated by this method are being used in preliminary in vitro screens for abiotic stress tolerance, such as heat, cold, salt and drought.

Characterizing in planta expression of G N-S, a soluble form of Tomato spotted wilt virus G S glycoprotein

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Phytopathology 101:S123
Tomato spotted wilt virus (TSWV) is an economically important disease of vegetable and ornamental crops worldwide. The virus is transmitted by thrips (Thysanoptera) in a circulative-propagative manner. Previous in vitro feeding experiments documented that the soluble form of TSWV GS, GS-S, interferes with virus acquisition and transmission by thrips. We expressed GS-S transiently in Nicotiana benthamiana to characterize GS-S behavior in planta and to determine if GS-S has potential for controlling virus transmission by thrips. The localization pattern of green and red fluorescent protein fusions (GFP or RFP) of GS-S were compared against the wild type GS (GN-Wt) and nuleocapsid (N) proteins. GS-S was distributed throughout the cytoplasm in 72% of cells examined and the distribution was similar to the GFP control. In contrast, GS-Wt displayed a distinct punctate pattern in 72% of cells examined. Co-localization experiments revealed that GS-Wt targets to the Golgi (96%) and GS-S and Golgi marker co-localization was observed in only 44% of cells evaluated. N displayed a complex localization pattern two days after agro-infiltration with protein accumulating in cytoplasmic foci of varying sizes and in small foci associated with the cell periphery suggesting that some N may localize to the plasmodesmata. We documented the localization of GS-S in plant cells and our findings indicate that generation of GS-S transgenic plants may be a viable TSWV control strategy.

Evaluation of the effects of soil moisture on the damage potential of Rotylenchulus reniformis on cotton

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Phytopathology 101:S123
A trial to reduce the risk of damage to cotton by Rotylenchulus reniformis was conducted in a 26 ha field in south Alabama in 2009 and 2010. The field was delineated into three management zones using apparent soil electrical conductivity (EC) and elevation, and the nematocides 1, 3-dichloropropene, aldicarb, oxamyl and abamectin were applied alone and in various combinations within each zone with an untreated control. Population densities of R. reniformis prior to nematicide treatment were 535, 1096 and 714 nematodes per 15cm3 soil for zones 1, 2 and 3, respectively. Zones 1, 2 and 3 averaged increasing volumetric water content (P < 0.1) of 0.138, 0.150 and 0.184 cm3/cm3 throughout the season. Evaluation of the interaction of R. reniformis population and soil moisture on cotton yields indicate that the driest zone, zone 1, was at the highest risk of yield loss and benefited with yield gains (P < 0.1) from higher rates of nematicides. Although zone 1 supported only half the initial R. reniformis population compared with zone 2, the combination of soil moisture and nematode stress in zone 1 resulted in a significant (P < 0.1) yield increase over the untreated control. The factor of water availability throughout the growing season should be considered in risk assessment when creating site-specific management zones for Rotylenchulus reniformis.

Pattern recognition feasibility of temporal dynamics of asian soybean rust backpropagation network

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Phytopathology 101:S123
The presence of free water on leaf surface and the average temperature during the wettest are the main factors for the occurrence and intensity of the progress of asian soybean rust. The aim of this study was to develop a neural network to characterize the weather favorability for the development of soybean rust in major soybean producing regions of Brazil. During the seasons 2007, 2008 and 2009 were carried out 22 experiments, which were collected meteorological data, plant development and disease severity. Data from 22 outbreaks were collected over three years. To quantify disease severity it was used the digital image processing by the software QUANT. For development of neural networks were used as input: the duration of leaf wetness (hours), the average temperature during leaf wetness, the first day that the disease was observed in each experiment and as output of the networks neural had foliar severity. The neural networks were developed in the Matlab Neural Network Toolbox, version 2009, using the backpropagation algorithm for training the networks, with 60% of the data was used for training, 20% of the data to test and 20% for validation. Choosing the best combinations of neurons was based on lower mean square error, mean prediction error and highest coefficient of multiple determination. The best combination of neurons showed the mean square deviation equal to 6.891 and the mean prediction error equal to 21.78% and determination coefficient of 0.842 in the validation scenarios.

Distribution and genetic variation of Thecaphora amaranthi in amaranth crop regions in Mexico

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Phytopathology 101:S123
Thecaphora amaranthi was reported in the amaranth crop in Tlaxcala State some time ago. Due to the importance of this amaranth smut for infecting the inflorescence ovaries and can replace the seeds, and besides the farmers behavior of each year exchanging amaranth seed, it was advisable to know the severity of T. amaranthi in different crop regions in Mexico. Due to the importance of this amaranth smut for infecting the inflorescence ovaries and can replace the seeds, and besides the farmers behavior of each year exchanging amaranth seed, it was advisable to know the severity of T. amaranthi in different crop regions in Mexico. During the seasons 2007, 2008 and 2009 were carried out 22 experiments, which were collected meteorological data, plant development and disease severity. Data from 22 outbreaks were collected over three years. To quantify disease severity it was used the digital image processing by the software QUANT. For development of neural networks were used as input: the duration of leaf wetness (hours), the average temperature during leaf wetness, the first day that the disease was observed in each experiment and as output of the networks neural had foliar severity. The neural networks were developed in the Matlab Neural Network Toolbox, version 2009, using the backpropagation algorithm for training the networks, with 60% of the data was used for training, 20% of the data to test and 20% for validation. Choosing the best combinations of neurons was based on lower mean square error, mean prediction error and highest coefficient of multiple determination. The best combination of neurons showed the mean square deviation equal to 6.891 and the mean prediction error equal to 21.78% and determination coefficient of 0.842 in the validation scenarios.
Primary postharvest evaluations of chemicals as inducers of resistance against Penicillium digitatum and Penicillium italicum on citrus fruits

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Phytopathology 101:S124

Various synthetic or natural compounds have been described as capable of controlling a large variety of plant diseases without showing a direct antimicrobial activity. Eight of these chemical inducers (potassium silicate (PSi), acetyl salicylic acid (ASA), β-amino butyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), sodium silicate (SS), hirpin (H), benzothiadiazole (BTH), and salicylic acid (SA)) were evaluated as postharvest treatments to induce resistance to citrus green and blue molds, caused by Penicillium digitatum and Penicillium italicum, respectively. For each pathogen, 30 µL of the chemical solution at least at three concentrations of active ingredient were placed, using a micropipet, in a rind wound. About 24 h later, 30 µL of conidial suspension were inoculated in an adjacent new wound. For each combination of chemical, concentration, and pathogen, 4 replicates of 5 oranges each were used per treatment. Treated fruit were incubated at 20°C and 90% RH for 7 days before determination of disease incidence and severity and pathogen sporulation. Four of the eight chemicals somewhat induced resistance to molds. On ‘Valencia’ oranges, PSi (300 mM) and BABA (0.3 mM) significantly reduced blue mold incidence by 50 and 37%, respectively, and INA (0.03 mM) reduced green and blue mold incidence by 26 and 58%, respectively. On ‘Lanelate’ oranges, BTH (0.9 mM) reduced green mold incidence by 20%. No significant effect was observed on disease severity and pathogen sporulation.

Detection of Ralstonia solanacearum in Hawaiian field soils and evaluation of composts for suppressing pathogen populations

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Phytopathology 101:S124

Bacterial wilt caused by Ralstonia solanacearum is the most important disease affecting edible ginger (Zingiber officinale) in Hawaii. Serious outbreaks began occurring in 1993 and large losses continue every year. R. solanacearum is present in soil and following crop failure, fields are abandoned and left unsuitable for ginger production for many years. While PCR detection of R. solanacearum in ginger tissue and water is straightforward, detection in soil has been problematic. DNA extracted from field soil rarely produced a R. solanacearum-specific PCR product even when collected from a field affected by bacterial wilt. We evaluated several enrichment-PCR methods and found them useful in determining the presence, viability and relative abundance of the pathogen. Soil sample extracts allowed enrichment for R. solanacearum over a period of 5 days, when routinely added R. solanacearum-specific PCR products after 24 or 48 hours, even from fields not planted with ginger for several years. In several cases, fields that tested positive using enrichment PCR were planted with ginger, with large losses in the ensuing crop. In one case, after initial disease onset, the grower applied high rates of a bran-enriched compost and avoided significant losses. We are performing additional greenhouse studies with enrichment PCR for evaluating various soil amendments, including compost and vermicompost preparations, for their ability to reduce pathogen populations in naturally infested field soil.

Corn yield components affected by controlling needle nematodes

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A field known to have needle nematodes present was selected as the site to determine plant responses that may relate to yield improvement (stand, plant height, tasseling date, ear length, ear weight, or number of rows of kernels per ear) in response to products that reduce nematode feeding damage. All plants in the center row of four rows were inoculated with 5000 Rhyzobius larvae (R. similis) per plant. In October, the number of seeds per plant was recorded. At harvest, the number of plants that were infested was recorded. All treatments resulted in significant final stand. All treatments resulted in similar final stand. No significant differences were observed among treatments for plant height, tasseling date or the number of rows of kernels per ear. Ear length increased when the treatment had a product that protects from early season nematode damage compared to Poncho 250 or Poncho 1250 for plants at V5 (P = 0.02), V6 (P = 0.081), and V7 (P = 0.069) and ear weight followed the same trend (P = 0.132 to P = 0.231). The ears of the smaller plants (V5 and V6) were closer in length and weight to the larger plants (V7) when a nematode control product was part of the treatment. Poncho/VO/TIVO and Poncho 1250/VO/TIVO had the highest total yield although it was not significant.

Control of Fusarium virgiliforme (sudden death syndrome) with a seed treatment

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Phytopathology 101:S124

Field results from 2010 testing in a soybean trial with severe symptoms of sudden death syndrome (SDS), causal pathogen Fusarium virgiforme, demonstrated that plots grown from seed treated with L1940-A exhibited little or no visual symptoms of SDS while adjacent plots without L1940-A as a seed treatment had severe symptoms. Later in 2010, green house experiments and a paper towel assay were completed to verify field observations. The paper towel assay resulted in the untreated control seed having 55% of the seeds showing F. virgiforme mycelial growth, while L1940-A at 0.05 mg ai/seed had 2.5%. Poncho/VO/TIVO + L1940-A at 0.1 mg ai/seed had 7.5% and L1940-A at the 0.15 mg ai/seed application rate had no seeds with mycelial growth. The green house trial resulted in significantly lower incidence and severity of SDS with treatments that contained L1940-A compared to the untreated control.

Integration of balanced crop nutrition and Chlorpyrifos in management of Coffee Berry Borer, Hypothenemus hampei (Coleoptera: Scolytidae) in Kenya

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Phytopathology 101:S124

The control of Coffee Berry Borer, Hypothenemus hampei (Ferrari) using cultural, chemical and biological strategies has remained a challenge. The cultural control is tedious and expensive, chemical control is ineffective when not applied on time because of unique life cycle of the borer while biological control has remained not very promising. To address the issue of Berry Borer control, use of balanced crop nutrition integrated with chlorpyrifos as foliar sprayed insecticide was assessed. Three different soil fertilizers were used as source of balanced crop nutrition. A field trial was laid out on a main coffee block planted with Ruiru 11, a coffee cultivar resistant to both the Coffee Berry Disease and Leaf Rust caused by Colletotrichum kahawae Waller and Bridge and Hemileia vastatrix Berkeley and Broome, respectively. Soil fertilizers; NPK 17:17:17, NPK 22:6:12 and Organic Compost (NPK 0.8:0.2:1.0) were applied in three different coffee sub blocks each with two plots, one sprayed with chlorpyrifos and unsprayed. The mean Berry Borer infestation for three years ranged between 1.16% and 19.39%. Except in one year, the Berry Borer row plot was maintained below 10% under any treatment, a level that was below the economical injury level. The study indicated that the three balanced soil applied fertilizers assessed, when integrated with Chlorpyrifos or applied alone controlled the Berry Borer.

Inheritance of resistance to Fusarium root rot in common bean

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Phytopathology 101:S124

Fusarium root rot (FRR) is a major disease of common bean. Knowledge of inheritance of resistance is important in developing resistant varieties. A 12 × 12 full diallel mating scheme generated 132 F1 progenies that were advanced to F3. Progenies were evaluated for resistance to FRR in a screen house. GCA effects were significant (P ≤ 0.01) for disease scores. SCA effects were not significant (P ≤ 0.05) in the F1, but were significant (P < 0.01) in the F3 indicating that resistance was governed by both additive and non-additive gene effects. Reciprocal differences were significant (P ≤ 0.01) reflecting influence of maternal effects. Non-maternal effects were strong in the F3, suggesting a complex form of cytoplasmic-genetic interaction. Average heritability of 0.44 was observed in each of the generations, indicating that epistasis was probably more influential than dominance of individual genes. Bi-modal distributions were characteristic of F3 distributions, and fit expected ratios for 2 or 3 segregating loci. Parent-offspring heritability estimates were moderate. Results indicated that resistant parents contain a number of different resistance genes that can be combined to produce strong and durable resistance. Lines MLB-49-89A, MLB-48-89A, RWR719 and Vuninkingi, with large and negative GCA effects contributed high levels of resistance in crosses and would be recommended for use in breeding programs.
The implications of non-crop hosts in the epidemiology of Tomato spotted wilt virus in the Solanaceae of Georgia

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The southern Georgia farmscape offers an environment highly conducive to disease and insect pressure. Although first reported in Georgia in 1986, Tomato spotted wilt virus (TSWV) was not considered a problem until the late 1990s. Since that time, farm gate losses in agricultural community have been devastating. The cultivation of several susceptible crops in close proximity to each other, the year-round availability of numerous, non-crop host species, the widespread presence of thrips vectors such as Frankliniella occidentalis and F. jasca, and the temperate environment conducive to TSWV infection cycles makes it control difficult. Observations focused on the farmscape have opened up new insights into the epidemiology of TSWV. The role non-crop hosts play in the epidemiology is crucial in understanding the overall disease cycle since inoculum sources of TSWV from these hosts could be a major part of the dynamic. A wide ranging screening of non-crop hosts of TSWV was begun in 2002 and continued for 9 years. During the study, over 100,000 weed samples were collected and screened for TSWV. Overall, approximately 5% were infected with TSWV. Seasonal infection levels and potential keystone species were identified and correlated with the associated crop’s infection levels. While the farmscape’s level of potential virus inoculum explained a portion of the disease dynamic, there is still much that is not known about the epidemiology of TSWV.

Detection of Fusarium oxysporum f. sp. canariensis and F. proliferatum from palms in southern Nevada

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Fusarium wilt is a serious vascular wilt disease on palms. Fusarium oxysporum f. sp. canariensis (FOC), primarily infecting Canary Island date palm (Phoenix canariensis), was identified from only one location in Las Vegas in 1999. The extent of the disease occurrence in southern Nevada is unknown. During 2009 and 2010, we received symptomotic frond samples collected from 10 F. canariensis and 1 California fan palm (Washingtonia filifera) in Las Vegas and Henderson. Samples when plated on PDA media rendered only growth of Fusarium species. Molecular identification was employed to determine their identities by amplifying, cloning, and sequencing a portion of genomic sequence diagnostic for FOC (GenBank Accession No. AF118442). Ten isolates obtained from F. canariensis were identified as FOC, while the isolate from W. filifera as F. proliferatum. Of the 9 FOC isolates, 5 had a DNA sequence 100% identical to that of FOC isolate 703C (Accession No. FJ895295.1), and remaining 4 had 100% identical to FOC isolate 2675A (Accession No. FJ895298.1). The two groups of FOC had similarly multiplexed with a Nad5 internal control assay targeting plant RNA. We also developed a multiplex one-step TaqMan real-time RT-PCR protocol for the detection and identification of CiLV-C in citrus plant samples. These new protocols were used to detect CiLV-C in citrus samples from Costa Rica and Panama.

Development and validation of a multiplex one-step RT-PCR for the improved detection of potyviruses infecting imported germplasm

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Foreign plant germplasm is a valuable source of novel genes not present in the international market. The previously developed universal potyvirus Nib primer pair (Nib2F and Nib3R) was used to detect all tested 40 potyvirus isolates representing at least 23 species. To increase speed and accuracy a multiplex one-step RT-PCR assay was developed using the Nib primer pair and an internal plant RNA control (Na5). The assay was validated and head-to-head compared with two other primer pairs (HP and CI) routinely used for potyvirus detection in diagnostic laboratories. Twelve different host species suspected to be infected with potyviruses were tested. The one-step multiplex RT-PCR assay produced the expected potyviruses amplicons of 350 bp as well as the host RNA internal quality control amplicon of 180 bp from all tested samples. The previously published potyvirus primer pairs produced inconclusive results for six (HP) or three (CI) of the virus isolates. PCR-amplified DNA fragments produced using the Nib primers were cloned and sequenced to verify the specificity of the assays. Eleven known potyvirus species and five previously uncharacterized potyviruses were identified. This multiplex one-step RT-PCR assay is well suited for the detection of known and unknown potyviruses in samples.
Proteome reference map for the soybean cyst nematode

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Phytopathology 101:S126

Soybean cyst nematode is the most destructive pathogen of soybean worldwide causing an estimated $2 billion in losses annually. Proteomic technologies are powerful tools to examine protein expression profiles as well as modification of proteins. We adapted these tools to investigate pathogenesis of SCN. We investigated and optimized protein extraction protocols and resolved several SCN proteins by two-dimensional gel electrophoresis. Three different protein extraction methods including phenol/ammonium acetate, thiourea/urea solubilization (lysis method) and trichloroacetic acid (TCA) solubilization were used to define their efficacy in separating SCN proteins by 2-DE. The phenol method showed higher protein resolution and spot intensity of all proteins compared with the other two methods. In addition, within the high-pI region, proteins resulting from phenol based extraction were well resolved and strongly detected. Protein spots obtained from the phenol method were subjected to matrix-assisted laser desorption/ionization time of flight mass spectrometry or liquid chromatography mass spectrometry to test their quality. In continuation of this project, we are also investigating differentially regulated proteins of infected root among resistant and susceptible soybeans. This information will help us to have a greater ability to identify the pathogen, understand its biology, host-pathogen interactions, and ultimately, to formulate improved disease management practices.

Virus-like particles of Maize rayado fino virus, Cucumber mosaic virus, and Lolium latent virus as chemical bio-conjugate substrates

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Phytopathology 101:S126

Plant viruses and virus-like particles (VLPs) are highly organized structures with unique chemical and physical properties. These properties can be exploited to generate nano-materials which have multiple uses. Novel VLPs can be produced by either genetically modifying the viral genome or by chemically attaching in vitro a variety of ligands, including fluorescent dyes, polymers, peptides and carbohydrates, to reactive groups on the viral capsids. Each of the subunits forming the VLPs potentially represents a platform to display functional groups, with control over spacing and orientation. Combining molecular biology, chemistry, and nanotechnology techniques, we explored the possibility to perform chemical modifications on the isometric VLPs of Maize rayado fino virus and Cucumber mosaic virus and the flexuous Lolium latent virus. The orthogonal reactivity and the suitability to serve as chemical bio-conjugate substrates was tested using cysteine side chain thiol-reactive probes including fluorescein-5-maleimide and lysine side chain amine-labeling reagents, including NHS-Fluorescein. Fluorescently labelled particles were analyzed by SDS-PAGE and by biotinylation assays demonstrating the feasibility of these reactive amino acids to be used in more advanced conjugation chemistries.

Effects of Coniothyrium minitans strains on viability of sclerotia of soybean white mold fungus

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Soybean white mold fungus (Sclerotinia sclerotiorum), preserves its sclerotia viable from three to several years, and degeneration of sclerotia are difficult. Therefore, alternatively antagonistic fungi have role mycorparasitising sclerotia. We compared the effectiveness of Coniothyrium minitans (CM) of Contans® WG and a newly isolated strain CMN09 from sclerotia of white mold infected plants, northeast research and demonstration farm, Nashua, IA in 2009. Freshly grown sclerotia on PDA were aseptically spread in 9-cm glass Petri dishes (20 per dish) containing sterilized (SS) and unsterilized (US) wet soil (80 g soil + 20 ml DSW). There were 4 treatments of each of (a) control, (b) CMN09 spray and (c) un inoculated controls. In aseptic conditions, 10 plates each of SS and US were spray-inoculated with pycnidiospores suspension of Contans and another 10 plates each of SS and US with pycnidiospores of CMN09. The spray suspension of each strain carried 2.2 × 10⁶ pycnidiospores/m². Five plates each from SS and US were un inoculated controls. Inoculated and un-inoculated plates were sealed with Parafilm, and a set of five plates from (a), (b) and (c) were incubated at 23°C, and another set at 3°C in 12 h fluorescent light. At 15 d interval for 90 days, 10 sclerotia from each treatment were sampled, surface sterilized and plated on PDA. Our observations showed that the sclerotia inoculated with CMN09 had low to 0% viability in SS compared with Contans and controls, indicating CMN09 may be more aggressive than Contans.

Elucidation of negative interactions between glyphosate and azoxystrobin and effects on Rhizoctonia solani severity under field conditions

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Roundup Ready sugar beet varieties and their wide use has led to an increase in glyphosate (GLY)-containing herbicides. Recent studies showed a reduction in efficacy of azoxystrobin (AS) when applied with GLY. Azoxystrobin is used to control Rhizoctonia solani AG 2-2 crown and root rot (CRRR) which can cause losses up to 50%. Interactions between GLY and AS might be of great importance since a combined application would be desirable to decrease application costs. The objective of this study was to test tank-mix applications of AS and GLY for their ability to control CRRR. The factorial experiment consisted of conventional and GLY based weed management practices combined with different AS applications timings. Tank-mix applications were applied as a banded or broadcast application. Reduced AS efficacy resulting from negative interactions between products was not observed. Data showed highly significant differences between conventional and GLY-based weed management practices with GLY increasing final stand and reducing CRRR. Weed competition in conventional plots led to a decrease in vigor resulting in smaller beets that could have been more susceptible to infections with CRRR. Comparing infected control plots of each weed management practice showed no significant differences for disease severity, but GLY treated plots showed a slight reduction (6%) in disease severity, verifying the absence of negative GLY effects.

A commercial extract of the brown seaweed Ascophyllum nodosum suppresses thrips in peppers, cucumbers and Hass avocados

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Phytopathology 101:S126

Thrips are found worldwide and cause damage to vegetables, fruits, and flowers. Some thrips are vectors of diseases such as Tomato Spotted Wilt Virus. A proliferation of thrips may also cause respiratory and skin irritation to workers. Effectively managing thrips with non-toxic materials has proven to be one of the most challenging aspects of natural pest control. An extract from the brown seaweed, Ascophyllum nodosum, harvested sustainably in Nova Scotia, reduced leaf deformation from Western Flower Thrips (Frankliniella occidentalis) based on leaf area measurements by 158% compared to the control on greenhouse-grown jalapeno peppers. Trials on greenhouse-grown cucumbers demonstrated a 54% reduction in the amount of leaf area damaged by thrips when plants were treated with A. nodosum extract compared to the water-treated control. Field-grown Hass avocado trees had 68% fewer Avocado Thrips (Scirtothrips perseae) per leaf compared to the control. This reduction in thrip numbers was not significantly different from abamectin; the most common chemical control for this insect in avocados. In addition, there were 87% less colonies of Persea mites (Oligonychus perseae) per leaf in the A. nodosum-treated trees compared to the control, which was also highly significantly different from abamectin. A. nodosum extract applications result in significantly less feeding damage by thrips on greenhouse-grown peppers and cucumbers and field-grown avocados.

Functional analysis of the Cucumber mosaic virus 2b protein

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Phytopathology 101:S126

Cucumber mosaic virus (CMV) is an economically important plant virus with a broad host range. The genome of CMV consists of three single-stranded, positive-sense RNA molecules, which encodes five proteins. The smallest one (110 aa) is the multifunctional 2b protein encoded by the RNA2. The 2b protein has a function in symptom induction, in viral movement, suppression of the immune response and in defense response. In our work the alanine scanning mutagenesis of the 2b protein was carried out. In the infectious clone of Rs-CMV the three-three consecutive amino acids of the 2b protein were replaced with alanine. The infectivity of the 37 mutant clones was tested on Nicotiana clevelandii plants in the presence of RNA 1 and 3. The infection was monitored by Northern analysis of the inoculated and the systematically infected leaves, and the stability of the mutants was verified by nucleic acid sequence determination after RT/PCR. The majority of the mutant viruses caused similar symptoms as the...
original Rs-CMV. In these cases the sequence analysis confirmed the stability of the mutations. In the case of six mutants symptoms were not observed and the presence of viral RNA was not detected in the non-infected leaves. In two cases the symptoms developed later, and in two further cases the test plants recovered. Our results will be discussed in relation with the known structure of the 2b protein. The project was supported by OTKA K75168 grant.

North Dakota populations of Leptosphaeria maculans are becoming more diverse

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Phytopathology 101:S127

Canola (Brassica napus) was introduced to North Dakota more than 25 years ago. Currently, >90% of the canola produced in the U.S. is grown in North Dakota. Blackleg, caused by the fungus Leptosphaeria maculans, became endemic in canola growing areas in mid 1980s. This disease is capable of reducing yield by >50%. In 1991, L. maculans isolates retrieved from infected plant tissues were determined to belong to pathogenicity groups (PG), 1 and 2. This classification was made using a set of three B. napus differentials, Quinta, Glacier and Westar. We collected isolates in 2004, 2007, and 2009 and phenotyped using the same differentials. Each of 195 isolates were inoculated on three sets of six plants from each differential by depositing 10 µl of a 10^7 pycnidiospores ml^-1 suspension on tiny wounds made with sterile needles on the cotyledons leaves. Reaction to inoculation was recorded 10 days later using a 0–9 severity scale. Prevalence of each pathogenicity group was estimated for each year and across all counties and Simpson’s diversity index was calculated. The index values of 0.2, 0.25, 0.3 and 0.46 in 1984–2001, 0.33, 0.32, 0.35 and 0.39 respectively indicated that PGs were dominant earlier but now it is more diverse and high proportion of new PGs (PGT, PG3 and PG4) have been introduced in ND. Presence of highly aggresive strain of L. maculans in high percentage and appearance of unknown group poses a serious threat of this disease to canola industry in North Dakota.

Population genetic structure of the fungus Leptosphaeria maculans in commercial canola (Brassica napus) fields in North Dakota

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Phytopathology 101:S127

Leptosphaeria maculans, is a fungal pathogen that causes blackleg or phoma stem canker of canola (Brassica napus) worldwide. In North Dakota, blackleg is one of the most devastating diseases of canola and the severity of the disease is increasing with occurrence of new pathogenicity groups. To study the genetic structure of the blackleg population in North Dakota, 276 isolates of L. maculans were collected from infected stubbles from commercial canola fields in ten North Dakota counties over a period of two years. Isolates within a county were considered a population. Populations were analyzed using six microsatellite markers. A total of 229 haplotypes were identified and high gene diversity (H = 0.454 to 0.682) was observed in the populations. High level of population differentiation (G’st = 0.149 to 0.683, p < 0.001) was observed among most of the pair-wise comparison between the populations. Analysis of molecular variance (AMOVA) also indicated that 84% of the genetic variation was found within the population, while the remaining 16% was found among the populations. Further analysis with more number of samples per location and additional microsatellite and minisatellite markers will help us better understand the genetic structure of L. maculans from North Dakota.

Interactive effects of temperature and wetness duration on infection parameters of Pseudoperonospora cubensis in cucurbit varieties

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Phytopathology 101:S127

Downy mildew of cucurbits caused by the oomycete Pseudoperonospora cubensis, is one of the most important diseases affecting cucurbits. A model based on a modified Weibull function was recently developed to quantify the effects of temperature and leaf wetness duration on sporangia germination and host infection. However, differences in host resistance on the predictable ability of the model have not been determined. Three cucurbit host types namely, cucumber (cv. Straight 8), cantaloupe (cv. Kermit) and squash (cv. Table Queen), were inoculated with P. cubensis and exposed to constant temperatures of 5 to 30°C during leaf wetness durations of 2 to 24 h in growth chamber experiments. Germination was assessed after each wetness period, while leaf area infected was assessed 5 days after inoculation. Germination and infection data were fitted to the model using nonlinear regression. Cultivar, temperature, wetness duration and their interactions significantly (P < 0.0001) affected germination and disease severity. For example, at 20°C, 15% leaf area infected was expected following 2, 4 and 8 h of wetness for Straight 8, Table Queen and Kermit, respectively. When temperature was increased to 25°C, 15% disease severity was expected following 3, 7 and 15 h of wetness for Straight 8, Table Queen and Kermit, respectively. Based on model parameters, host based nomograms were developed to predict infection risks based on combinations of temperature and leaf wetness duration.

Microsatellite profile of Puccinia psidii in Hawaii and South America

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Phytopathology 101:S127

Rapidly mutable microsatellite markers were used to assess the genetic relationships among rust populations from South America and Hawaii. Such genetic markers provide reliable genetic information, allowing inferences about potential sources of pathogen introduction. The hypothesis is that Puccinia psidii populations from South America are distinct from the rust populations that became established in Hawaii. The eight microsatellite loci analyzed revealed 14 multilocus genotypes (MGs) within the 22 P. psidii isolates. Isolates collected on different hosts in South America (Eucalyptus spp., Psidium guajava, P. araca, Syzygium jambos, S. cuminii, Myrciaria cauliflora, and Eugenia uniflora) presented distinct MGs. In contrast, all rust isolates collected on nine myrtaceous hosts in the Hawaiian Islands (Metrosideros polymorpha, M. excelsa, Eugenia koalauensis, Rodomyrthus tomentosa, Myrtus communis, S. samaranganse, M. quinquinervia, S. cuminii, and S. jambos) were of only a single unique MG. The MG comprising all isolates from Hawaii is distinct from the MGs found in South America so far, suggesting that the Hawaiian isolates did not come directly from South America. Isolates from California, Florida, Central America and Caribbean must be analyzed to better understand potential relationships with pathogen dispersion to Hawaii.

Multilocus genotypes indicate selection by host in Puccinia psidii populations from Brazil

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Phytopathology 101:S127

Population genetic approaches were used to determine the genetic structure of the rust fungus Puccinia psidii in Brazil, believed to be the pathogen putative center of diversity. Ten microsatellite markers were used to examine the amount and distribution of genetic variability within 148 rust isolates obtained on seven Myrtaceae hosts across a wide geographic area. Analysis of molecular variance indicated no genetic differentiation among isolates from different geographic locations, and high differentiation between isolates from different hosts (97%, P > 0.001). Principal coordinate plots, also indicated high degree of genetic differentiation among isolates collected on different host species, revealing five major groups. The Neighbor Joining tree also clustered the rust isolates on five groups, based on host of origin. The high proportion of repeated multilocus genotypes within each host, combined with high values of IA and rD, and low values of FIS, indicates high rate of clonal reproduction. The haplotype MJ-Network also supported the hypothesis of host selection and clonal reproduction. Microsatellite data indicates potential selection by host and high rate of clonal reproduction on P. psidii population from Brazil. Phylogenetic studies are underway to check the possible existence of cryptic rust species.

Resistance to the stem rust ‘Up99’ race group in spring wheat landrace accessions from the USDA-ARS National Small Grains Collection (NSGC)

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Phytopathology 101:S127
Germlasm collections of crop landraces conserve genetic diversity and may contain novel sources of disease resistance. New sources of stem rust resistance are needed to help manage the *Puccinia graminis* f. sp. *tritici* ‘Ug99’ race group (TTKS lineage). From 2007 to 2011, nearly 3000 spring wheat landraces from the NSGC were screened for resistance in eight field seasons at the Kenya Agricultural Research Institute, Njoro, where the *Ug99* race group is endemic. Most accessions were selected for screening based on resistance to U.S. races, but approximately one-third were chosen randomly or by geographic origin. Accessions showing resistance in one season were re-tested in Njoro and screened as seedlings against race TTKSK. Resistant accessions were also screened with molecular markers diagnostic for *Sr2*, *Sr24*, and *Sr36*. To date, field results are available for seven of eight seasons and 165 accessions have shown resistance in more than one season. With 57% of the marker screening completed to date, 8 and 11 of the resistant accessions were positive for markers associated with *Sr36* (wmc477) and *Sr2* (csSr2), respectively. Less than 5% of the field-resistant accessions that were negative for *Sr2* were susceptible to TTKSK as seedlings, suggesting potential new sources of adult plant resistance (APR). To genetically characterize the resistant accessions, association mapping studies are being conducted and bi-parental mapping populations are being developed.

**Evidence for multiple fungicide resistance in field populations of *Venturia inaequalis***

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Apple scab caused by *Venturia inaequalis* is the most economically important fungal disease of apple in the eastern United States. Site-specific fungicides remain the most effective tool for scab management in commercial apple production but they are prone to development of resistance. All scab management programs in the region therefore recommend rotating fungicides with different chemistries to mitigate resistance development. Analysis of a large sample of *V. inaequalis* population (4841 isolates from 131 orchards in 14 states) tested for resistance to doline, the sterol demethylating inhibitor (DMI) myclobutanil, and the quinone outside inhibitor (QoI) thioxy邻baso indicated a highly significant (*P < 0.0001*) incidence of isolates with resistance to the three fungicides. The odds of an isolate resistant to myclobutanil also being resistant to doline were more than twice (*odds ratio = 2.31; 95% confidence interval [CI] = 1.95 to 2.75*) those of a sensitive isolate, and a DMI-resistant isolate was ≥2.5 times as likely to be quantitatively resistant to the QoI fungicide (*odds ratio = 2.69; 95% CI = 2.30 to 3.16*) compared to a DMI-sensitive one. Moreover, the incidence of isolates with resistance to at least two of the three fungicides was significantly higher (*P < 0.0001*) in orchards subjected to a total of ≥20 or ≥15 applications of a DMI or a QoI fungicide, respectively, by the time of the survey. These results suggest a reassessment of the current strategies for resistance management is required.

**Spatial and temporal patterns of insect damage and aflatoxin contamination in corn at pre-harvest**

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Ear-feeding insect damage and aflatoxin contamination are the key impediments to corn yield and quality under warm climatic conditions worldwide. A series of experiments have been conducted to examine the contribution of insect damage to aflatoxin contamination. To assess the spatial and temporal patterns, aflatoxin contamination and insect damage was sampled twice with a 4-wk interval before harvest in 2008 and 2009 using a grid-sampling method. The feeding damage by each of the ear/kernel-feeding insects (i.e., corn earworm/fall armyworm damage on the silk/cob, and discoloration of corn kernels by stink bugs), and maize weevil population were assessed at each grid point with five ears. The spatial distribution pattern of aflatoxin contamination was also assessed using the harvested corn samples from each sampling point. The aflatoxin level was not correlated to the number of ear/kernel-feeding insects but correlated to stink bug-discolored kernels in the corn fields in 2008, whereas the 2009 data showed the opposite. The maize weevil infestation, stink bug-damaged kernels, and aflatoxin levels also showed a clustered distribution pattern with a strong edge effect across the fields. The comparison of the results from the two-sampling dates showed that temporal pattern of aflatoxin levels was only changed in 2009, but not in 2008. A 4-wk- and cob-feeding insect damage assessment and their damage in relation to aflatoxin accumulation and its management strategies will also be discussed.

**Effect glucorafano isolated of broccoli florets on the germination of *Rhizopus stolonifer* spores**

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Rot caused by *Rhizopus stolonifer*, is one of the most severe postharvest diseases of strawberry, the strategy most used to control disease, is the pre-and post-harvest treatment with fungicides, but their use is increasingly restricted due to public awareness of hazardous waste in the fruits. Glucosinolates are natural products containing nitrogen and sulfur and its antimicrobial activity has been shown in other research. For this work, we collected fruits of strawberry, with symptoms of rot: from them it was isolated and identified the fungus *Rhizopus stolonifer*. The spores of the pathogen were placed on PDA with different glucorafanone concentrations (1.54, 0.92, 0.46, 0.15, 0.02 y 0 mg μL−1) isolated from broccoli florets. We measured spore germination until the control treatment show the highest percentage of germination. The median lethal concentration was 1.01 mg μL−1 and the concentration that completely inhibited the germination of spores was 2.16 mg μL−1.

**The effect of imazalil on Colletotrichum gloeosporioides isolated from avocado (Persea americana) fruit**

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Colletotrichum gloeosporioides is the causal agent of anthracnose in avocado, leading to production losses of almost 20%. Nowadays, the most useful strategy for the control of the disease is the fungicide treatment; however, in Mexico there are only a few compounds authorized for postharvest use. For this reason, the effect of imazalil on the mycelium growth and spore germination in vitro was evaluated in this study. The fungus was isolated from avocado fruits of Uruapan, Michoacan. Twelve concentrations of imazalil (0.01, 0.1, 1.0, 5.0, 10, 50, 100, 200, 400, 600, 800 and 1000 ppm) were evaluated in vitro. The fungicide doses corresponding to each treatment were previously dissolved in the culture medium. A completely random experimental design was used. For the evaluation of the mycelial growth, 5 plates per treatment were used, and for the spore germination 100 spores were evaluated for each treatment. The variance analysis showed that the factors under study had a statistically significant effect (*Pr = 0.0001*) on the mycelial growth and spore germination. The effect of imazalil was observed from the 5 ppm dose, which provided an efficacy of 79.86%, while from 10 ppm up to 1000 ppm the efficacy reached 100%. In addition, it was found that the LC 50 of imazalil for the control of C. gloeosporioides is 0.79 ppm. Imazalil proved to be excellent for controlling C. gloeosporioides in vitro. However, in Mexico it has not been authorized for its use on avocado.

**Survey of *Erwinia amylovora*, causal agent of fire blight, from apple and pear orchards in Utah for streptomycin resistance**

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Phytopathology 101:S128

Fire blight caused by the bacterium *Erwinia amylovora* results in millions of dollar in losses worldwide. It is the most important disease problem for apple and pear growers in Utah. Currently the only effective management strategy is the application of streptomycin. In 2006, resistant isolates were detected in an apple orchard for the first time in one Utah county. To determine the distribution of resistant isolates and the level of resistance, isolates collected in 2006, 2007, 2010 and 2011 from apple trees across the state were tested for resistance to streptomycin. Each isolate was initially screened at 0, 100 and 1000 ppm of streptomycin. Bacteria were quantified with a spectrophotometer and 100 microliters were spread on LB agar. A whole was punched from the agar and the streptomycin solution was pipetted into the well. The plates were evaluated after 24 hours for bacterial growth. A bacteria-free zone around the whole was observed for sensitive isolates that was not seen with resistant isolates. Isolates sensitive at 100 ppm were tested at lower concentrations and isolates resistant at 1,000 ppm were exposed to higher concentrations. The majority of resistant isolates were found in Utah County where most apple and pear orchards are located. Resistant Isolates tolerated at least 100,000 ppm of streptomycin.
Susceptibility of mesquite species to powdery mildew in Arizona  
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Phytopathology 101:S129

Mesquite (Prosopis sp.) is a popular tree in landscapes in Arizona because of its drought tolerance and attractive growth habit. Powdery mildew has been observed from late summer until early spring on mesquite leaves. It has been identified on several mesquite species based on morphological and microscopic comparison to herbaceous specimens. To determine the susceptibility of different mesquite species to powdery mildew, 175 mesquite trees from eight species were surveyed for the presence of powdery mildew from fall 2008 until early 2009 on The University of Arizona campus in Tucson, AZ. Leaves were inspected under a dissecting scope for the presence of powdery mildew. Only the North American mesquite species were inspected under a dissecting scope for the presence of powdery mildew. No powdery mildew was observed on P. alba, P. nigrum, P. chilensis, P. pubescens and P. chilensis × flexuosa in the vicinity of infected trees. The powdery mildew affects the aesthetic value of severely infected trees but seems to have little effect on long term tree health.

Root-knot nematode species in golf course greens in the Western U.S.A.  
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Phytopathology 101:S129

Root-knot nematodes (Meloidogyne sp.) have been a problem in golf course greens in the Western U.S.A. for several years. Symptoms include irregular yellow patches in the turf grass that may eventually die. The problem with managing populations of Meloidogyne sp. was exacerbated after the only chemical product available for control on turfing was removed from the market in 2009. To develop alternative management strategies, it is important to determine which species of Meloidogyne are present in golf courses. Root-knot nematodes collected from over 100 golf courses in the western states were extracted from the soil using a mist extractor. Juvenile (J2) root-knot nematodes were identified using a dissecting scope. Individual J2s were cut in half in extraction buffer and lysed. PCR was conducted using primers amplifying the D2-D3 region of the 28S gene as well as the ITS region. Thus far we have detected, five known and two unidentified species. The known species identified are M. nasus, M. minor, M. citriwoodi, M. marylandi and M. graminis.

Microorganisms and antifungal properties associated with noni (Morinda citrifolia) fruit and fermented juice in Hawaii  
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Phytopathology 101:S129

Noni (Morinda citrifolia), a medicinal plant grown in Hawaii and other Polynesian regions, is reportedly therapeutic for diabetes, high blood pressure, and certain types of cancer. Noni fruit often produce fermented juice that affects storage factors affecting juice quality and juice quality. We studied the occurrence of microorganisms during fermentation and also report on antifungal properties of pasteurized juice exudates in vitro bioassays. Firm, yellow, mature noni fruit were held in sealed glass jars for up to 40 days at 22°C. Juice exudates were analyzed weekly for microbial populations and chemical properties. Puree of fresh soft fruit was also analyzed. Bioassays consisted of PDA plates spread with sporangia suspensions of fungal pathogens and spotted with juice, puree, or sterile distilled water. Bacterial populations did not differ from 0 to 35 days, but were highest at 42 days. Macrocirrhelosporium t. sp. circinelloides, a fungus consistently isolated from fermented juice samples, had populations that peaked at 14 days. Fresh noni puree was microbe-free or low in microbial populations. Total soluble solids (% TSS) were highest in fresh noni (9.8), then significantly decreased after 14 days (5.7) storage. In bioassays, the TSS of noni puree or juice with antifungal activity against several pathogens of tropical fruit crops was determined: activity was absent at 4 to 5% TSS; intermediate at 6% TSS; and highest at 7% TSS or greater.

The plant growth-promoting rhizobacterium Bacillus cereus AR156 induces resistance in Arabidopsis thaliana and tomato  
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Phytopathology 101:S129

We previously isolated a rhizobacterium Bacillus cereus AR156. It was shown to significantly protect tomato against bacterial wilt caused by Ralstonia solanacearum and root-knot disease caused by Meloidogyne incognita. Here, we investigate the ability of AR156 to promote plant growth and induce the systemic protection of Arabidopsis thaliana and tomatoes in greenhouses against bacterial spee disease caused by Pseudomonas syringae pv. tomato DC3000. Compared to mock-treated plants, AR156-treated ones showed an increase in biomass and reductions in disease severity and pathogen density in the leaves of Arabidopsis. The defense-related genes PR1, PR2, PR5, and PDF1.2 were concurrently expressed in the leaves of AR156-treated plants, suggesting simultaneous activation of the salicylic acid (SA)- and jasmonic acid (JA)/ethylene (ET)-dependent signaling pathways by AR156. The above gene expression was faster and stronger in plants treated with AR156 and inoculated with DC3000 than that in plants only inoculated with DC3000. Similarly, AR156 also increased the average biomass of the tomato and elicited induced systemic resistance (ISR) against DC3000. In the further study, we will investigate how AR156 can simultaneously activate these pathways, which will be instrumental in improving the application of AR156 to plant protection. Guo JH is the corresponding author.

Development of microsatellite markers for population genetic analysis of Waitea cirtina var. cirtina  
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Waitea cirtina var. cirtina is an emerging pathogen of turfgrass in North America. It causes brown ring patch of turfgrass in golf courses and amenity areas. Recent attention is on its almost countrywide distribution despite having been reported for the first time in U.S.A. in 2007. To better understand the population biology of W. cirtina var. cirtina, we have isolated eight promising microsatellite markers from an enriched genomic library. Seventy clones were arbitrarily selected from the library and sequenced. Of these, 48 (68%) contained microsatellites. Twenty-seven of the 48 candidate microsatellite loci shared sequence similarity with one another or had their repeats too close to the end of the sequence. Primers flanking the repeat region were designed for the remaining microsatellite loci and were initially sequenced on five isolates of W. cirtina var. cirtina from different geographic regions. A total of 10 (62%) microsatellites were polymorphic. Two of these alleles were unique per isolate and had a minor band. Eight candidate microsatellite loci are further being characterized with additional 35 isolates of W. cirtina var. cirtina; two W. cirtina var. zeae and three of Rhizoctonia solani.

Industry-wide assessment of methyl bromide alternatives and sting nematode management in Florida strawberry  
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Florida strawberry research continues to focus on a co-application approach of different fumigants, herbicides, and other alternative tactics to achieve pest control efficacy and crop growth response similar to that of methyl bromide soil fumigation. With increasing cost and reduced availability, growers are increasingly transitioning to a diverse array of methyl bromide alternative tactics, one of which has not been by large volume for field research. Since 2009, over 50 commercial strawberry fields with recurring histories of nematode problems have been studied to characterize differential responses of fumigant alternatives using estimates of relative strawberry yield. Relative strawberry yields were determined from ground truth survey of plant size categories and with NDVI (Normalized Difference Vegetation Index) using GreenSeeker® (NTech Industries; Ukiah, Ca) optical sensors. Ground truth survey of plant size categories was well correlated with NDVI estimates of canopy cover. Overall, the methodology is being used to provide growers guidance and quantitative performance data on alternatives to methyl bromide soil fumigation for nematode management on a farm by farm and industry-wide basis.

Large scale demonstration trialing of drip fumigants in Florida strawberry  
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Phytopathology 101:S129

Three drip applied soil fumigants were evaluated as alternatives to methyl bromide for pest control efficacy and strawberry yield enhancement in five grower field trials. Soil treatments included chisel applied methyl bromide (50%) chloropicrin (50%) (288 – 320 lb/a), compared with the drip applied
fumigants including, metam sodium (75 gpa), Pic Clor 60EC (300 lb/a) and Telone Inline (35-48 gpa) applied and evaluated with either one or two drip tapes per bed at either of two farm locations. Assessments of plant growth included differences in plant size, health, vigor, and yield with treatments arranged as a completely randomized block design with at least 4 replications per treatment. In general, a significant drip tape effect (1 vs 2) was observed with strawberry yield and plant growth improvement with methyl bromide chloropirin (a horticultural effect). Strawberry plant growth and yield was significantly improved 10 to 15% when drip fumigants were delivered with two drip tapes per bed compared to one. With two drip tapes per bed, improvements in strawberry plant growth occurred as a result of a horticultural effect (improved water and nutrition) and also as a fumigation effect (improved movement and bed distribution of the fumigant). Additional research is required to validate the fumigate, horticultural, and economic benefits of the drip fumigants and additional drip tape.

Endospore forming bacteria indigenous to landscape planting beds and their inhibition of Rhizoctonia solani
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Phytopathology 101:S130

Indigenous endospore forming bacteria were collected from root samples of bedding plants grown in established commercial landscape beds. One hundred twenty-nine bacterial strains associated with 14 species were identified using FAME analysis. All strains were evaluated for in vitro inhibition of damping off disease caused by the fungus Rhizoctonia solani. The strongest in vitro inhibition was achieved by soluble exudates in cocultivation was observed with strains belonging to 6 species: Bacillus cereus, Bacillus pumilus, Bacillus thuringiensis, Lysinibacillus sphaericus, Bacillus amyloliquefaciens, and Bacillus subtilis. Most strains that inhibited Rhizoctonia growth in cocultivation also inhibited growth via volatile compounds. All 129 strains were evaluated for ability to protect impatient plants from subsequent challenge infection by Rhizoctonia solani. Certain strains of endospore forming bacteria also enhanced plant growth. It is apparent from this study that this bacterial community associated with roots of plants within established planting beds produces soluble antifungal compounds, volatile antifungal compounds, and enhance plant growth. In developing biological control, it may be a more practical approach to promote or enhance a natural, multifaceted community of Bacillus strains within our planting beds.

Characterization of a novel satellite RNA associated with natural population of Cucumber mosaic virus (CMV) in Wisconsin snap bean fields
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Selected strains of Cucumber mosaic virus (CMV), in addition to three viral genomic RNAs, contain a small linear, single stranded RNA called satellite RNA. Previously, CMV sat-RNAs have been reported to have dramatic influences on symptom expression, ranging from symptom attenuation to increased symptom severity. Further, the appearance of CMV sat-RNA is very common under greenhouse conditions, but is considered less common in agricultural and natural ecosystems. We have identified a sat-RNA naturally occurring in fields of CMV-infected snap beans in Wisconsin. A specific product of approximately 350 bp was amplified by RT-PCR with specific primer pairs. The amplified product was cloned and transformed into the pGEM-T Easy vector and E. coli DH5a cells, respectively. Three colonies were selected and sequenced bidirectionally and analyzed. BLAST analysis confirmed these small RNAs as CMV sat-RNAs with 339 nucleotides. Sequestered comparisons showed that an RNA isolated from a snap bean strain and a CMV sat-RNA from a tomato strain diverged by 15 nucleotide substitutions, three deletions and one insertion. Therefore, it appears this newly emerged WI-sat-RNA is a unique CMV sat-RNA. Field-collected, CMV-positive snap bean plants often showed severe CMV symptoms when co-associated with sat-RNA. Emergence of this CMV outbreak beginning in 2000 in the upper Midwest and Northeastern U.S., is associated with the emergence of the WI-sat-RNA in the CMV population.

Optimization of RNA isolation and qRT-PCR strategies to monitor microbial gene expression in soil
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The use of quantitative reverse transcriptase real-time PCR can lead to a better understanding of the microbial processes occurring in the rhizosphere and in situ action of biocontrol mechanisms. However, it is hindered by various technical factors when analyzing environmental soil samples. In this study, the efficiency of RNA isolation protocols, soil parameters such as clay content, and quantification approaches (absolute or relative) were investigated for their effects on microbial gene transcript quantification. Pseudomonas sp. LBUM300, a biocontrol agent of interest producing 2,4-diacetyl phloretin (DAPG) and hydrogen cyanide (HCN), was used as a model organism to target phlD and hcnC, involved in DAPG and HCN production, respectively. Time course experiments were conducted by inoculating soils with different quantities of LBUM300, and for the first time, a recently developed artificial exogenous spike-in RNA control (myfIC) was used for relative quantification. When comparing the RNA isolation protocols, the quantity of isolated RNA and detected gene transcripts were significantly affected by clay content, RNA isolation protocol and the interaction between both factors. Absolute and relative quantification lead to similar transcriptional trends for both genes, however the relative method proved more reliable for detection of low transcript numbers. In conclusion, recommendations are made as to which technical approaches seem well adapted for quantifying microbial gene transcripts in soil.

Use of plastic and spray mulches to manage insects vectoring plant viruses
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An investigation was conducted looking into ways to repel aphids and thrips from pepper plants to prevent virus infection has been carried on since 2008 in Kern. Silver reflective mulch has been used as a method to repel aphids and thrips from various crops to prevent virus transmission. It is often used on tomatoes, melons, and peppers to prevent virus infection such as tomato spotted wilt virus (TSWV), tobacco mosaic virus (TMV), CMV and others. Other colored plastic mulches have been shown to increase plant size and yield. The main objective of this study was to determine which plastics mulches besides silver reflective mulch could repel aphids and thrips to prevent virus transmission. Another objective was to determine if a more cost effective spray on mulch could be used to repel aphids and thrips. Lastly determine what effect these different mulches have on plant growth and yield.

Homologous recombination and the invasion of a new plant host by the phloem encoded bacterium, Xylella fastidiosa
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Phytopathology 101:S130

Homologous recombination plays an important role in the structuring of genetic variation of many bacteria; however its importance in pathogen evolution has yet to be established. We investigated the involvement of recombination in the shift to a novel host (mulberry) by the plant pathogenic bacterium Xylella fastidiosa. X. fastidiosa infects xylem and causes leaf chlorosis in many economically important plant species, including Pierce’s disease of grapevines. Mulberry leaf scorch was identified about 25 years ago in the eastern U.S. and since that time it has spread to California. Previous genetic analysis separated the mulberry isolates from the 4 recognized subclasses. Comparison of a newly sequenced genome of a mulberry strain with pre-existing genome data showed that this form originated by massive recombination between two of the subspecies, Xylella fastidiosa subsp. fastidiosa and Xylella fastidiosa subsp. multiplixa, resulting in a genome consisting of roughly an equal mix of the two subspecies. The extensive recombination involved in the origin of the mulberry type, combined with a very low level of within-type genetic variation, suggests the host shift was achieved after strong selection acted on genetic variants created by inter-subspecific homologous recombination. These data show that invasion of mulberry by X. fastidiosa provides a compelling example of the importance of recombination in the shift of a pathogen to a new plant host.

Identification and differentiation of gall midge species from West Africa
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The African rice gall midge (ArRMG), Orseolia oryzivora Harris and Gagné (Diptera: Cecidomyiidae), is a major biotic constraint to lowland rice production in West Africa. Studies have shown that resistance of rice varieties
to ARGM differs markedly from one location to another. This is probably due to genetic differences between the gall midge populations at different locations. An understanding of the genetic variation amongst the population of the Orseolia species is necessary for breeding programmes aimed at effective development of cultivars with durable resistance to ARGM in West Africa. Gall midge larvae and pupae were collected at random from various localities in lowland and irrigated rice ecologies in Nigeria, Mali, Burkina Faso, Cameroon and Sierra Leone. The insects were then processed for DNA analyses. PCR analysis of genomic DNA from the insects was carried out using sequence characterized amplified regions (SCAR) primers developed by Novlene et al., 2006. Cluster analysis revealed two major insect genotypes (OSG-1 and OSG-2). OSG-1 was further divided into two subgroups (OSG-1a and OSG-1b). All the three reference insects (Orseolia bonzii, Orseolia nwanzei and Orseolia oryzivora) were genetically distinct. While Orseolia bonzii and Orseolia oryzivora were genotyped as OSG-1b along with other twelve insects, only Orseolia nwanzei was genotyped as OSG-2. The study revealed the population structure of gall midge species in different rice ecologies of West Africa. Key words: Rice varieties, Orseolia oryzivora, Orseolia bonzii, Orseolia nwanzei, PCR analysis, SCAR markers, population structure, differentiation.

An in vitro baiting assay for recovery of Phytophthora ramorum from waterways

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The current protocol used by the USDA Forest Service for recovering Phytophthora ramorum from potentially infested waterways is by in situ baiting with root-tip and rhododendron tissues for 1 to 2 weeks. Filtration has also been used for recovery by passing aliquots from a 1-liter sample of water through membrane filters with 3- or 5-µm pores. We compared these recovery methods to an in vitro baiting assay that used both intact leaves and leaf pieces of rhododendron. For all three assays, pathogen detection was attempted by nested PCR and isolation on selective medium. For the in vitro assay, 900 ml of stream water was collected in 100-ml aliquots and placed in a 1-liter Nalgene screw-top bottle; the water was baited with 20 leaf pieces and one whole, non-wounded leaf. The bottle was held at 18 to 20°C in the dark for 3 days; baits then were removed, rinsed in distilled water, and blotted dry. Pathogen detection was initiated immediately for the leaf pieces, but whole leaves were incubated in a moist chamber for up to 7 days to allow lesion development. P. ramorum was recovered by the in vitro assay in 12 of 18 samples collected from streams where P. ramorum had been recovered by the current protocol and by filtration in 10 of the 18 samples; inoculum densities in these samples ranged from 1 to 130 cfu/liter. The in vitro assay showed promise as an alternative to the current protocol, but additional evaluation is needed before recommending this new recovery method.

Bacterial and fungal pathogens associated with diseased oil palm (Elaeis guineensis) plants in Pamol Plantations, Cameroon, Central Africa

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Oil palm is an important crop in Cameroon because of income generated from palm oil and palm kernel oil. Pests and diseases are important production constraints to this crop. In April 2010, ten oil palm fields and two nurseries in Limenja and Kokundi divisions of Pamol were surveyed for fungal and bacterial pathogens infecting them. In each field, stem, root and leaf samples of 4 symptomatic plants having chlorosis, stem rot, brown leaf spots and 1 asymptomatic sample were collected randomly and their tissues placed on Potato-pentachloronitrobenzene, Potato Dextrose Agar and Nutrient Agar. Pure cultures raised from single colonies were identified based on morphology and biochemical assays. Out of 110 samples collected, 38.2% had fungi which include Fusarium oxysporum, F. verticillioides, Botryodiplodia theobromae, Curvularia lunata, Colletotrichum gloeosporioides, Trichoderma sp., Cercospore sp., Cercospora sp., Cladosporium sp. and 21.8% bacteria; Bacillus cereus, Bacillus subtilis, Penicillium sp. and Xanthosomas sp. Their frequency of occurrence were 63.0%, 13.6%, 4.5%, 27.2%, 40.9%, 9%, 4.5%, 18.0%, 2.0%, 54.5%, 45.4%, 4.5% and 4.5%. Mixed occurrence of microorganisms was noted in all diseased samples, with 21.0% having 4, 33.0% having 3, 11.9% having 2, Latent infections with BT, Curvularia lunata and Bacillus subtilis was noticed in 5 out of 22 symptomatic plants. Purified culture of each microorganism was inoculated singly and in various combinations onto 4 months old asymptomatic plants to determine their pathogenic effects.

Temporal and spatial spread of Cucurbit downy mildew in the eastern United States

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Phytopathology 101:S131

Dynamics of cucurbit downy mildew, caused by Pseudoperonospora cubensis, in the eastern U.S. in 2008 and 2009 were investigated based on disease epidemics monitored in 24 states as part of the Cucurbit downy mildew IPMPIPE monitoring program. The mean season-long rate of temporal disease progress was 1.4 new cases per day across the two years. Disease progress was slow during the spring and early summer and did not enter its exponential phase until mid June. The median nearest-neighbor distance of spread of new disease cases was approximately 110 km, with about 15% of the distances being >240 km. Considering all cucurbits, the epidemic expanded at a rate of 10 km per day during recent years. Disease outbreaks were spatially aggregated and the extent of spatial dependence was up to 1,000 km. Results suggests that disease outbreaks in the Great Lakes and mid-Atlantic regions may be due to the spread of P. cubensis sporangia from outbreaks of the disease near the GA/SC/NC border rather than from overwintering sites in southern Florida. Space-time point pattern analysis indicated strong (P < 0.01) evidence for a space-time interaction and a space-time risk window of about 3 to 5 months after first disease outbreak and 300 to 600 km was detected across the two years. Results support the hypothesis that infection of cucurbits by P. cubensis appears to be an outcome of a contagion process and factors occurring on a large spatial scale (~ 1,000 km) facilitate the spread of the disease in eastern United States.

Systemic nematocidal activity of fluensulfone against the root-knot nematode Meloidogyne incognita on pepper

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Phytopathology 101:S131

Fluensulfone, a new nematocide belonging to fluoroalkenyl group, has proved to be very effective in controlling root-knot nematodes Meloidogyne spp. by soil application. We evaluated the systemic activity of the compound on M. incognita on peppers. Root application of fluensulfone via soil-drenching showed only slight nematode control activity when applied 4 but not 10 days after inoculation. A single foliar spray of peppers with a fluensulfone solution at 3.0 g/l prior to inoculation reduced the galling index by 80% and the number of nematode eggs by 73–82% of the control. The reduction in these parameters by fluensulfone was much higher than those obtained by oxamyl or fenamiphos at the same concentration. This activity was also observed when the plants were sprayed 21 days prior to inoculation. A series of experiments suggested that foliar spray with fluensulfone prior to inoculation reduced the nematode invasion. In fact, the number of invading juveniles in the roots of seedlings sprayed with fluensulfone was lower than that in the roots of the control plants. However, foliar spray after inoculation did not inhibit the nematode development inside roots. These results suggest that fluensulfone applied to the foliage may translocate to the roots and affect the nematode invasion into roots. Fluensulfone may be used as foliar application for root-knot nematode control.

Myrothecium roridum tode and its toxin shows potential for management of water lettuce

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Phytopathology 101:S131

The management of aquatic weeds with synthetic chemical herbicides is not only expensive but is beset with myriads of environmental and health implications. The current trend in weed management includes the use of host-specific mycoherbicides and phytotoxins. The effects of Myrothecium roridum (IMI 394934) and its phytotoxin on water lettuce (Pistia stratiotes) were examined. The fungus isolated from diseased water hyacinth plants in Badagry Creeks, Lagos, Nigeria was made into a suspension (1 × 106 spores/ml) with 0.01% tween 80 in sterile distilled water and the pathogenicity on fresh non-diseased P. stratiotes was investigated. The pathogen was re-isolated from the dead inoculated test plant in conformity with Koch’s postulates. Detached leaves of P. stratiotes were infiltrated with 10 µL (30%) crude fungal toxin produced in potato sucrose broth and monitored for 7 days post toxin infiltration. Visual examination of disease development revealed a necrotic symptom in the P. stratiotes leaf on day 5 and 100% mortality was also observed on day 37 post fungal inoculation. The phytotoxin caused foliar symptom on the leaves with an average severity index (ASI) of 3.0 (> 15% vein discoloration) on day 3 and 6.0 (above 75% vein discoloration) on day 7 post toxin infiltration. These results indicate that M. roridum and its phytotoxin are effective in causing lethal effects on P. stratiotes hence can be considered as potential herbicial agents, suitable for use in the management of P. stratiotes.
Survival potential of Phytophthora infestans: sporangia in relation to meteorological factors

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Phytopathology 101:S132

Assessment of meteorological factors coupled with sporangia survival curves may enhance effective management of potato late blight, caused by Phytophthora infestans. We utilized a non-parametric density estimation approach to evaluate the cumulative probability of occurrence of temperature and relative humidity conducive for late blight outbreak at the potato production field site at Presque Isle, ME. Sporangia survival probabilities were also computed based on microclimatic data. The influence of solar radiation on sporangia survival potential was also computed, based on the modified survival model. The joint distribution of temperature and relative humidity were similar among years, and favorable for late blight outbreak. Sporangia survival duration and frequency coincided with the potential period for pathogen infection during the cropping cycle. Sporangia survival probability (SSP) ranged from 0–64%, but had variable frequency and temporal changes during the cropping cycle. Analyses of SSP showed that 5–10% of cropping cycle is associated with 48–64% survival probabilities. High humidity and low temperatures were correlated with low solar radiation and increased risk for disease outbreak. By modeling periods of elevated survival potential in diverse locations and production regions, precise forecasts and disease controls can be optimized. P. infestans survival curves and climatic variables can be utilized in predictions of late blight on potato tubers and for better disease controls.

Corn and soybean yield responses using sedaxane, a new seed treatment experimental fungicide from Syngenta

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Phytopathology 101:S132

Azoxystrobin resistant isolates of Cercospora sojina isolates are a conidia germination assay conducted in agar plates amended with azoxystrobin and sulhydrazinamid acid (SHAM). In this study, a mycelial growth inhibition method was evaluated to determine sensitivity levels of isolates to azoxystrobin and to discriminate sensitive and resistant isolates. The mycelial growth inhibition of 3 azoxystrobin sensitive and 3 resistant isolates was evaluated on potato dextrose agar amended with azoxystrobin (0, 0.001, 0.01, 0.1, 1 and 10 mg/L) and SHAM (50 mg/L) to establish the sensitivity of the isolates (ED50 values). Mycelial inhibition was obtained using the conidia germination assay. The mycelial growth inhibition method was effective in determining the azoxystrobin sensitivity of C. sojina isolates and confirmed the results of the conidia germination assay. Mycelial growth inhibition methods can be used in future azoxystrobin resistant studies to discriminate resistant and sensitive isolates. This method is also less labor intensive than the standard conidia germination assay.

Usefulness of a high-throughput transient expression system to test virus-derived genetic constructs for resistance against Grapevine fanleaf virus


Phytopathology 101:S132

Grapevine fanleaf virus (GFLV) causes fanleaf degeneration disease of grapevines. Since resistance to GFLV has not been identified in wild or cultivated grapevines, varied genetic constructs derived from GFLV have been generated with the aim of conferring resistance in transgenic grapevine rootstocks. To reduce the time and expense involved in the production and testing of transgenic grapevines for resistance to GFLV, a high-throughput approach has been developed for evaluating the anti-viral potential of candidate constructs by utilizing an Agrobacterium tumefaciens-mediated delivery system to achieve transient expression in Nicotiana benthamiana, a systemic host of GFLV. This approach allows for screening putative resistance constructs over a considerably shorter time frame than testing transgenic grapevines. Replicated experiments have indicated that many of the constructs can reduce virus titres in agroinfiltrated plant tissues, as shown by enzyme-linked immunosorbent assays and semi-quantitative RT-PCR, with differential levels of anti-viral activity observed among constructs. In order to test whether the transient approach is an accurate predictor of the anti-viral competency of constructs, transgenic N. benthamiana have been produced and utilized in resistance screening assays. Results from comparative resistance evaluations using the transient expression system and stable N. benthamiana transformants will be discussed.

Resistance to race TTKSK of Puccinia graminis f. sp. tritici in tetraploid wheat

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Phytopathology 101:S132

A group of races of Puccinia graminis f. sp. tritici in the TTKS (or Ug99) lineage possess broad virulence to wheat cultivars worldwide, and only a few genes in adapted cultivars have resistance to these races. In attempts to identify new stem rust resistance genes effective against race TTKSK, we evaluated cultivated and wild tetraploid wheat (T. turgidum ssp.) for resistance to TTKSK and other races with broad virulence. A high frequency of TTKSK resistance at the seedling stage was observed, as 395 (21% of 1882) accessions exhibited low infection types. Studies to determine the genetic basis of TTKSK resistance at the seedling stage revealed that resistance in tetraploid wheat is conferred mostly by single genes. Additional resistance genes effective against races TRTTF and TTTTF were also identified. Three hundred seventy tetraploid accessions were evaluated for resistance in the field screening nurseries of Debre Zeit (Ethiopia) and St. Paul (MN). One hundred fourteen accessions exhibited resistant to moderately resistant responses to stem rust in both nurseries. Fifty-two accessions susceptible to TTKSK, TRTTF, and TTTTF at the seedling stage were resistant at the adult stage. The accessions may possess adult plant resistance. Since all these tetraploid species share the same genome as durum wheat, resistance genes could be easily transferred to durum wheat by conventional breeding approaches.

Pesticidal activities of Hyiptis suaveolens in pest management

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Phytopathology 101:S132

Fresh, mature and healthy plants of Hyiptis suaveolens were collected in Akungba Akoko, Ondo State, Nigeria. The essential oil of the plant was extracted sequentially through solvent extraction methods using n-hexane, diethyl ether and methanol. The Methanolic extract was further prepared to obtain concentrations of 100%, 75%, 50%, 25%, 10%, 5%, 4%, 3%, 2%, and 1% which were tested for pesticidal activity against cultures of selected pest species of storage crops and mosquitoes. The experiment revealed the high insecticidal capability of H. suaveolens through repellent and fumigant application and 100% mortality of Stipitella oryzae, Stipitella zeamae and Callosobruchus maculatus within 10 seconds at the 50% methanolic extract in the contact treatment.

Fungicide application on disease resistant wheat: Is the response what you would expect?

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Phytopathology 101:S132

Foliar fungicides are often applied to susceptible winter wheat varieties in Oklahoma to protect yield potential when disease pressure is high. Typically fungicides are not applied to disease resistant wheat varieties. Here we analyze disease rating and yield data from five variety trials planted in 2005, 2008, 2009 and 2010 at two locations in Oklahoma. Trials were planted in a

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randomized complete block design (four replications) with a split plot arrangement of treatments with wheat varieties as the main plot and fungicide treatment (sprayed or non-sprayed) as the subplot. Varieties ranged from susceptible to resistant to leaf rust, which was the predominant disease. Four of five trials had increased mean yields when all varieties were combined. The mean percent yield (bu/acre) increase with all trials and varieties pooled was 6.0 (p = 0.001). The sixty-four of twenty-eight varieties had mean yield increases when trials were combined, with variety 2174 having the greatest positive yield response to fungicide (19.4%) and TAM 401 having the greatest negative response (~18.4%). These observations suggest that fungicide applications on disease resistant varieties may benefit yield and indicate that yield increases may be variable dependent.

Sedaxane, a new experimental active ingredient from Syngenta for seed treatment use

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Phytopathology 101:S133

Sedaxane is a new experimental fungicide active ingredient developed by Syngenta Crop Protection for seed treatment use. It belongs to the chemical class of Pyrazole-Carboxamides and inhibits Succinate-Dehydrogenase, a central enzyme of the fungal respiration chain. Due to its activity spectrum and biokinetic properties, the AI is especially suited for seed treatment use. It is active against many important soil- and seed-borne diseases like Ustilago spp., Tilletia spp., Monographella nivale, and Rhizoctonia spp. Examples from greenhouse and field tests are presented to exemplify the level and spectrum of activity of Sedaxane.

The development of a specific Real-Time TaqMan for the detection of Clavibacter michiganensis supsp. michiganensis

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Phytopathology 101:S133

The detection of Clavibacter michiganensis supsp. michiganensis is of great economical importance. This seed born pathogen, with quarantine status, is the causative agent of bacterial cancer in tomato and causes economic losses in commercial tomato and seed production. The currently used detection methods all have drawbacks. The development of a new detection method was therefore still needed. IISH-NL used AFLP® to find specific fragments present in all selected Clavibacter michiganensis supsp. michiganensis isolates. Only one single fragment of interest was found. The fragment was partial coding for a gene producing a protein two-component system sensor kinase. This gene was used to develop the specific RZ_Ptsk MGB based TaqMan®. The developed RZ_Ptsk TaqMan® was tested and compared with existing Real-Time PCR assay’s on a collection of 67 isolates. The RZ_Ptsk TaqMan® was the only assay correlating 100% with a pathogenicity test on tomato plants. Several IISH_NL and international labs are using the RZ_Ptsk TaqMan® for their own specific purpose. Until today no false negative or false positive isolates have been reported.

Effect of fungicides on the control of postharvest diseases in papaya fruits

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Phytopathology 101:S133

In the state of Colima, Mexico there are about 1,500 hectares of papaya across a broad range of elevations in the state of Sonora, Mexico. The current study evaluated the influence of elevation on the composition of aflatoxin-producing fungi in maize fields of Sonora, Mexico at varying elevations: A three year study

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Phytopathology 101:S133

Aflatoxin contamination of maize, a critical staple of billions, by Aspergillus flavus is a recurrent problem in the tropics and subtropics. Maize is produced across a broad range of elevations in the states of Sonora, Mexico. The current study evaluated the influence of elevation on the composition of aflatoxin-producing fungal communities associated with maize and the stability of those communities over time. Fungal isolates (1,230) belonging to Aspergillus spp. were recovered from field-soils previously cropped to maize in 27 locations across 300 km at elevations ranging from 6 m to 2,100 m in the summers of 2006, 2007 and 2008. Fungal community structure was characterized for the A. flavus L strain isolates (846) with vegetative compatibility analyses utilizing nitrate non-utilizing auxotrophs. In total, 125 vegetative compatibility groups (VCGs) were detected; VCG composition varied greatly from year to year. Many VCGs that were very common in one year were rare or not detected in other years. Only 10% of VCGs were detected in each of the three years studied while 63% of VCGs were detected
only in a single year. These results suggest that dynamics of communities of aflatoxin-producing fungi resident in agricultural fields are complex resulting in rapid shifts in composition.

**Potential organic substrates for soil application of Microspora amaranthi and Phomopsis amaranthi**

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Phytopathology 101:S134

Microspora amaranthi and Phomopsis amaranthi, applied as bioherbicides, have shown utility controlling common waterhemlock (*Amaranthus retroflectus*) and redroot pigweed (*Amaranthus rudis*), two weeds having resistance to multiple herbicides. However, unless specially formulated, the survival, infectivity, and efficacy of these fungal pathogens as foliar sprays is constrained by environmental factors. Field and growth chamber trials, conducted in Western Illinois, examined BioApt Granular Peat-Based Microbe Carrier and corn stover as potential substrates for these organisms as soil-applied PRE- or POST-emergent products. BioApt or corn stover infested substrates were distributed evenly over the soil surface of 8.9 cm² pots at a rate of 1.5 g per pot after planting 50 weed seeds (PRE) or when weed seedlings reached the 1-2 true leaf stage (POST). Micropot trials, at two field locations, evaluated BioApt applied at 40 g per 30 cm² microplot. Bioherbicide PRE- and POST-emergence effectiveness in trials was determined by counting weed emergence, rating disease incidence, and measuring plant biomass (G10 to 14 DAT). Our results indicate that PRE or early POST granular applications of these bioherbicide organisms have some activity but more work is needed to develop effective application parameters. Determining an economical and effective substrate, allowing these organisms to be soil-applied as PRE or POST emergent products could benefit the possible commercialization of these bioherbicide organisms.

**A preliminary account of the sanitary status of *Prunus* species in the National Clonal Germplasm Repository**

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Phytopathology 101:S134

The USDA National Clonal Germplasm Repository (NCGR) at the University of California, Davis is recognized as one of the richest sources of stone fruit material in the U.S. The repository maintains more than 1600 *Prunus* accessions representing 94 species collected from around the world. However, the phytosanitary status of the NCGR *Prunus* collection has never been systematically evaluated. We have now completed the first comprehensive testing for virus and virus-like diseases for a small part of the collection. A total of 223 trees representing 185 different cultivars of *Cherry*, *Almond*, *Peach*, *Apricot*, and *Plum* were sampled. qRT-PCR and/or RT-PCR analysis was used to test for 13 different viruses, 2 viroids, and a phytoplasma. Though the majority of these trees were asymptomatic, all tested pathogens were detected in the vast majority of *Prunus* accessions. This included *Apple chlorotic leaf spot virus*, which had never previously been reported in California. The infection rate of the trees ranged from 66% infection with *Prunus necrotic ringspot virus*, to 0.5% phytoplasma infection. The PCR amplicons from positive samples were sequenced to analyze the molecular variability between isolates.

**The effect of seasonal changes on grapevine leafroll associated viruses’ titer and distribution in the grape**

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Phytopathology 101:S134

A quantitative virus survey was performed for two years with three months interval to monitor the seasonal virus titer profile of grapevine leaffoll associated viruses (GLRaV) in infected grapevines. The viruses included in the study were GLRaV-1, -2, -3, -4, -5, and -9 and GLRaV-2 Redglobe strain (GLRaV-2RG). A time course experiment was performed in which sixty five grapevines varieties previously determined to be multiply infected with different grapevine viruses were selected as the starting material. The samples were collected in three month intervals in May August November and February over two years. From May to November, leaf petioles and in February dormant grapevine cuttings were collected. The samples were tested by using One-Step qRT-PCR. The data showed that in general the titer of viruses associated with leaffoll disease was higher in the period from November to February. The lowest titer was observed in the period from May to August. In addition a distribution study was performed in which samples from 5 different locations of each GLRaV-infected vines were selected and tested. The results showed that these viruses were not equally distributed within the same grapevine plant.

**Proposed guidelines for sample processing and downstream detection of grapevine viruses**

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Phytopathology 101:S134

This study sets up guidelines for efficient sample processing and downstream detection of grapevine viruses. Different methods for homogenization, RNA extraction and qPCR based detection of grapevine RNA viruses have been evaluated. Semi-automated and automated homogenization techniques were compared to process samples from grapevine petioles and cambial tissue. Four different high throughput automated nucleic acid extraction platforms were compared with the RNeasy Plant Extraction kit for their capacity and efficiency for extracting RNA from grapevine infected tissues. The RNAs prepared from each extraction platform was then used as template for a comparative analysis of quantitative PCR (qPCR) by One-Step qRT-PCR, Two-Step qRT-PCR and low density arrays (LDA) detection. This study showed that a thorough homogenization of grapevine tissues using the Tissue Lyser as well as DNase digestion of the purified RNA prior to cDNA synthesis improved the virus detection and yielded the lowest Cq values in qRT-PCR. Comparison of different RNA extraction methods showed that methods implementing the magnetic bead-based technology were superior to other methods compared. Comparing different qPCR detection methods, One-Step qRT-PCR showed the lowest Cq values for the same sample tested and was able to detect higher number of positive samples compared to Two-Step qRT-PCR and LDA.

**Comparative expression analysis of genes encoding pectin methyltransferase enzymes in *Phytophthora* infected during infection of *Solanum tuberosum***

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Phytopathology 101:S134

*Phytophthora* infests is the causal agent of late blight disease of potato, which causes billions of dollars of crop loses annually. Current research efforts on late blight and *P. infestans* focus on understanding the mechanism of pathogen infection. While it is accepted that the pathogen infects hosts through the use of specialized structures, most importantly the appressorium, it is also known that the force of this structure alone is not sufficient to penetrate the host’s rigid cell wall. As a result, investigations are now being conducted to examine the specific role played by cell wall degrading enzymes (CWDE’s) which are thought to be involved in *P. infestans* infection development. Previous research showing variability in the relative fold expression (RFE) of several CWDE’s in *P. infestans* grown in vitro has prompted this study. We report on the expression profiles of all *P. infestans* genes previously identified by bioinformatic approaches as belonging to a gene family with known pectin methyltransferase (PME) activity. The gene expression patterns of these genes were first evaluated in qualitative and quantitative real-time PCR, using total RNA samples obtained at six different time points during the infection process on potato plants. Data from the in planta assays indicate that there are significant differences in the expression levels of the targeted genes at different times during the infection process, suggesting that PMEs might play a key role in *P. infestans* pathogenicity.

**First report of *Phytophthora* ramorum infecting *Trachelospermum Jasminoides* in Oregon**

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Phytopathology 101:S134

*Phytophthora* ramorum can reportedly infect >125 plant species, many of which move through the nursery trade. In April 2010, *P. ramorum* was detected infecting *Camellia* plants growing in a nursery in Washington County, Oregon. During the delineation survey to determine the extent of the *CWDE’s* which are thought to be involved in *P. ramorum* infection development. Previous research showing variability in the relative fold expression (RFE) of several CWDE’s in *P. ramorum* grown in vitro has prompted this study. We report on the expression profiles of all *P. ramorum* genes previously identified by bioinformatic approaches as belonging to a gene family with known pectin methyltransferase (PME) activity. The gene expression patterns of these genes were first evaluated in qualitative Real-time PCR, using total RNA samples obtained at six different time points during the infection process on potato plants. Data from the in planta assays indicate that there are significant differences in the expression levels of the targeted genes at different times during the infection process, suggesting that PMEs might play a key role in *P. ramorum* pathogenicity.
onto the semi-selective medium PARP. One *Phytophthora* isolate was obtained from the symptomatic leaves; this isolate was morphologically identical to *P. ramorum*. Results from the ITS and Elicitin qPCR tests verified the presence of *P. ramorum* DNA in the leaf tissue. As required by federal protocol, a subsample of DNA was sent to the USDA/APHIS/PPQ Molecular Diagnostic Laboratory for official confirmation. In June 2010, USDA/APHIS/PPQ/MDL confirmed our identification of *P. ramorum*. To our knowledge, this is the first report of *P. ramorum* infecting star jasmine in Oregon.

**Blackstain root disease effects on foliar nutrients, chlorophyll content, and internodal growth in ponderosa pine**

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Phytopathology 101:S135

We excavated root systems from four codominant non-symptomatic (based upon crown characteristics) and seven symptomatic mature ponderosa pine in the Lassen National Forest in NE California. Trees were approximately 100 years old. Data were obtained on foliar nutrients N, P, Ca, Fe, Mn, B, and Zn. Chlorophyll a and b concentrations were also determined, along with fascicle and needle length, needle and fascicle fresh and dry weights, and fascicle number and flush length. Root infection was quantified by measuring stain circumference on major lateral roots, expressed as percentage of total sampled root circumference. Non-symptomatic trees had two times greater flush lengths and more retained fascicles per flush than symptomatic trees. Chlorophyll a and b concentrations peaked during the second year after needle appearance in all trees and were only slightly higher overall in non-symptomatic trees. Foliar Zn, Ca, and Mn concentrations were highest in non-symptomatic trees while no such trends were evident for N, P, K, and Mg. Foliar Ca and Mn increased with needle age, which was up to seven years at this location. Symptomatic trees had a range of 23 to 66 percent of sampled root circumference involved with blackstain vs 0-4 percent for non-symptomatic trees. Given the amount of root infection observed in symptomatic trees, foliar nutrient and chlorophyll contents are conserved, although infection effects are most striking when expressed as flush lengths and amount of retained needles and fascicles.

**Sensitive detection and discrimination of *Xylella fastidiosa* subsp. *paucapauca*, causal agent of citrus variegated chlorosis**

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*Xylella fastidiosa* subsp. *paucapauca* (Xfp), a xylem-limited bacterium and select agent, causes citrus variegated chlorosis (CVC). The pathogen has spread through South and Central America, but is not in the U.S. CVC could significantly impact citrus producing U.S. states. A method for early, accurate and sensitive detection of Xfp in plant tissues is needed by plant health officials for inspection of products from quarantined locations, and by extension specialists for detection, identification and management of disease outbreaks and reservoir hosts. Two sets of specific PCR primers and probes, targeting Xfp genes for fimbriin and perilipase iron-binding protein, were designed. A pair of conserved primers targeting the cobalamin synthesis protein gene was designed to detect all possible *X. fastidiosa* strains. All three primer pairs and probes were validated in *silico* against published sequences. PCR products were cloned and sequenced for confirmation. Primer sets XfCVC_fimi1 (110 bp product), XfCVC_pibd4 (82 bp product) and Xf_csp6 (92 bp product) detected as little as 1 fg of plasmid DNA carrying *X. fastidiosa* target sequences at cycle threshold (Ct) values of 27.92, 30.19 and 29.55, respectively. These PCR assays are useful for *X. fastidiosa* detection, discrimination, diagnosis and quantification, and for applications in breeding programs, biosecurity and microbial forensics.

**Population structure and mating system of the faba bean pathogen, *Didymella fabae* (anamorph: *Ascochyta fabae*), in Syria**


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Ascochyta blight of faba bean (*Vicia fabae*) is caused by the fungal pathogen *Didymella fabae*. The fungus has a bipolar mating system and the sexual stage has been reported from artificially infected experimental plots in Syria. Eighteen sequence-tagged microsatellite (STMS) markers were developed to investigate the genetic structure among a sample of 96 isolates from three geographic locations (sub-populations) in Syria. Genetic linkage analysis of the STMS detected 8 linkage groups and clone-corrected data set of 63 isolates based on a randomly-chosen set of 8 unlinked markers were analyzed. AMOVA indicated small (10%) but statistically significant (P<0.01, F<0.05) genetic differentiation among sub-populations but the entire sample of isolates was assigned to a single genetic population using a Bayesian clustering algorithm. A PCR-based mating type assay was used to determine mating type and a 1:1 ratio of mating types could not be rejected in each sub-population and among all sampled isolates. Multilocus gametic disequilibrium was estimated with index of association within sub-populations and among all isolates using 8 STMS markers and the mating type marker. The null hypothesis of random mating was rejected within each sub-population and among all isolates. These results question the role of ascospores as a significant source of inoculum for this disease in Syria.

**Population genetic structure of *Phytophthora cinnamomi* associated with *Phytophthora root rot of avocado* (*Persea americana*) within California**

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*Phytophthora* root rot (PRR) of avocado (*Persea americana*), caused by *Phytophthora cinnamomi* (Pc), is the most serious disease of avocado worldwide. The pathogen population genetic structure of Pc is usually associated with low genotypic diversity and the dominance of a single mating type with axenic reproduction. However, no population level studies have specifically been conducted in the avocado growing regions of California (Ca). Therefore, we used AFLP markers to investigate pathogen diversity of Pc from 16 groves from the Northern and Southern avocado growing regions. Additional isolates from other countries and hosts were also used for comparative purposes. Three distinct clades were found based on UPGMA analysis of 22 polymorphic loci; one clade contained only isolates from Southern Ca, one clade contained isolates from both Northern and Southern Ca, and the last clade contained mostly non-Ca isolates from additional hosts. From the Ca avocado populations, a total of 16 genotypes out of 169 isolates were found. The results indicate significant population structure in the Ca avocado Pc population, low genotypic diversity consistent with asexual reproduction, and potential evidence of movement of clonal genotypes between the two growing regions. Since two main clades were found among Ca isolates of Pc, these results may have implications for rootstock breeding against Pc if differences in virulence or aggressiveness occur within these two clades.

**Endophytic associations and production of mycotoxins by the *Aspergillus* section *Nigri* species**


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The filamentous fungi of the *Aspergillus* section *Nigri* (black aspergilli) are considered plant pathogens of maize (*Zea mays*) and peanuts (*Arachis hypogaea*) where they can cause similar disease symptoms as *Fusarium* vascular pathogens, such as seedling blight. However, the main concern with black aspergilli is their ability to produce carcinogenic mycotoxins such as ochratoxin A (OTA) and the fumonisins (FB1, FB2, and FB3). Our preliminary work indicated that these fungi were endophytes of maize. The first aim of our research was to provide evidence of endophytic associations between maize using yellow and red fluorescent protein-labeled strains of *A. niger* var *niger* (yfp) and *A. carbonarius* (rfp). The identities of the fungi were determined using a repetitive-sequence-based DNA method. This study revealed that both *Aspergillus* species had similar host colonization patterns in maize as endophytes. The second aim was to determine mycotoxin production by these species. We determined the ability of 167 field isolates to synthesize OTA, FB1, FB2, and FB3 in maize kernels as natural substrate and that of the species isolated, only 10% of *A. niger* var *niger* strains produced OTA. However, almost all *A. carbonarius* isolates produced the fumonisins. Our results are the first to indicate that black aspergilli associated with field maize in the United States are able to produce carcinogenic compounds, and are potential threats to human and domestic animal health.
PVX-M3 – A deviant pepper isolate of Potato virus X  
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Potato virus X (PVX) and cucumber mosaic virus (CMV) co-infection was  
determined on pepper showing typical mosaic symptom in Hungary in 1976.  
PVX was separated from CMV by thermal treatment of crude extract of the  
virus infected tobacco passed to healthy plant. This PVX-M3 isolate did not  
differ from common PVX strains based on the symptoms caused on different  
test plants. A polyclonal antiserum prepared against this isolate reacted  
similarly to the known isolates of PVX (PVX-G, PVX-NyH). The purified  
virion of PVX-M3 and PVX-NyH isolated from potato has been stored. In the  
autumn of 2009 proved to be both isolate infectious. Similarly of the previous  
investigation of polyclonal antibodies (Loewe) PVX-M3 and PVX-NyH  
isolates gave a strong reaction, but ADGENE monoclonal antibody only  
reacted with PVX-NyH isolate. Negative results with monoclonal antibody  
indicated an unusual property of the coat protein. Coat protein gene of this  
isolate was cloned and sequenced. We observed 4 amino acid changes in the  
N-terminal variable part of the protein compared to other isolates. The second  
two of which are separated by one amino acid induced changing in the protein  
structure resulting loosing hydrophybity and an alpha-helix disappearing.  
Thus, the epitope recognized by the monoclonal antibody has been changed.  
This may explain why that monoclonal antibody failed to detect this virus  
 isolate. The project was supported by NKTH-TECH-09-A3-2009-0210 and  
TAMOP-4.2.1-B-09/1/1KMR-2010-0005 grants.

Morphological-molecular characterization of Phytophthora, Pythium and  
Phytophthora on intensive crops in Buenos Aires – Argentina  
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Surveys of Oomyctes associated to diseases of ornamental, vegetable,  
soybean and fruit crops were carried out from 2006 to 2010 in cropping areas  
of Buenos Aires Metropolitan Area. Seventy five isolates were obtained from  
symptomatic roots, stems and leaves plated in selective media and transferred  
to CMA, PDA and V8. Isolates of Aspergillus niger fum6 and P.  
chamaehyphon fum19, encoding a cytochrome P450 monooxygenase, was absent.  
In contrast, PCR products for all eight gene targets were detected in the  
65 non-toxicigenic strains, suggesting that there may be structural or regulatory  
defects in one or more genes essential for FB5 biosynthesis in those strains.  
The occurrence of multiple genotypes among non-toxicigenic A. niger strains  
rises questions regarding the ecological significance of FB5 production, and  
may be useful in designing biocontrol intervention strategies for reduction of  
fumonisin contamination of grapes and other fruits.

Quorum sensing directly controls the Hrp regulatory cascade and the  
Gac/Rsm signal transduction pathway in the gall forming Pantoaea agglomerans  
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Gall induction by Pantoaea agglomerans pv. glyosophila (Pag) on glyosophila is  
hrp-dependent. Disruption of the quorum sensing (QS) genes pagI and  
pagR significantly reduced gall size and expression of the hrp regulatory  
genes hprX, hprS and hprL in planta as determined by qRT-PCR. Transcription of the  
QS and hrp regulatory genes was reduced by inactivation of IAA or cytokinin biosynthetic pathways. The interrelationship between the QS, hrp operon, cluster, the Gac/Rsm cascade and virulence has been investigated by gel shift experiments and the effects of mutants on gene expression using qRT-PCR in planta, colonization and gall formation in glyosophila. Gel shift electrophoresis indicated that PagR directly binds to the hrp regulatory genes in a C4-HSL-dependent manner to putative lux box in their promoters. Moreover, PagR also binds to a putative lux box located in the gacA promoter, which encodes the response regulator of the GacS/GacA two component system. The Gac/Rsm cascade, which controls the activity of RsmA, was investigated by studying the effects of gacS, gacA, rsmB and csrD mutants as well as overexpression of rsmB and rsmA for all the above-mentioned parameters. Overexpression of RsmA reduced virulence whereas its elimination abolished gall formation. Results presented suggest that PagR is a central regulatory factor that controls virulence through direct activation of the Hrp regulon and control of RsmA activity through the Gac/Rsm cascade.

Protection of cucumber diseases by using hot water extract from spent  
substrate of edible mushrooms  
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Since the impact of agrochemicals on environmental contamination, human  
health and pesticide resistance, alternative technologies for pest management  
are being investigated. One technology is the use of elicitors that are released  
from the mycelia of fungi. In this study, the mycelia of edible mushrooms that  
are prevalent in the spent substrate were used to induce defense mechanism in  
cucumbers. Plants were treated with autoclaved water extract from spent  
substrate of edible mushroom (AWESMS) of Lophyphum decastes or Pleurotus eryngii by  
dipping the first true leaf, and inoculated with the target pathogen after 1  
week. Results showed that AWESMS of L. decastes significantly reduced  
diseases by Colletotrichum orbiculare and Podosphaera xanthii in more than  
80%, but this effective result was not observed with Cynespora cassiocola.  
The AWESMS of P. eryngii was effective against C. orbiculare but not  
against P. xanthii. When cucumber plants were grown in pots containing  
a mixture of autoclaved spent substrate of L. decastes and soil (1:2, v/v), a  
disease reduction of over 70% was observed with C. orbiculare. The  
AWESMS of L. decastes showed no antifungal activity against C. orbiculare  
and a significant increase of expressions of chinatin B and β-1,3-glucanase 24 h  
after pathogen inoculation was observed. The use of spent substrate for  
disease control may offer a new technology for the recycling and management  
of waste from mushroom cultivation.

Grafting of a commercially important but bacterial wilt susceptible  
tomato variety with disease resistant rootstocks for open field production  
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Bacterial wilt of tomato caused byRalstonia solanacearum is a serious  
disease worldwide including Florida and Virginia. Grafting bacterial wilt  
susceptible scions with new and traditional resistant rootstocks is a
possible approach for managing the disease in open field production. Field and greenhouse studies were conducted at Quincy, FL, and Painter, VA during 2009-2010 that evaluated seven new tomato rootstocks (Jjak kung, Cheong gang, RST 105, RST 106, BHN 998, BHN 1053, BHN 1054) and a traditional rootstock (Hawaii 7998) as possible resistant sources for grafting a commercially popular, but bacterial wilt susceptible tomato variety BHN 602. Greenhouse studies in R. solanacearum inoculated potting medium confirmed that all the rootstocks except RST 105 were moderately or highly resistant to the pathogen. Field studies showed that the plants grafted on to Cheong gang, RST 106, BHN 998, BHN 1054, and Hawaii 7998 exhibited the highest level of disease resistance in FL and VA. Field studies also indicated that all these rootstocks had significantly higher yield compared to un-grafted and the self-grafted entries under high disease pressure. The findings from these trials illustrate that bacterial wilt can be effectively managed by grafting with resistant rootstocks. Although grafting will increase the cost of transplants, it will be economically beneficial in fields with a history of bacterial wilt, ensuring the sustainable production of the crop.

Marker-assisted selection improves the efficiency of bioprospecting and results in the recovery of novel biocontrol bacteria

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In the search for microbial products, large collections of microorganisms are screened for novel and effective isolates. In order to more efficiently recover and exploit, a greater variety of plant-associated bacteria as biopesticides, we developed a multivariate sampling and marker-assisted selection strategy. In doing so, we quantified the relative effects of different sampling and selection factors on the diversity of recovered bacteria, showing that variation in all factors could result in the recovery of distinct genotypes as defined by amplified ribosomal DNA restriction analyses (ARDRA). The efficiency of bioprospecting was improved by focusing phenotypic characterization solely on representative ARDRA-defined genotypes. Subsequent sequencing and phenotypic analyses revealed that our marker-assisted selection strategy led to the recovery several rare and, to date, poorly characterized genera of plant-associated bacteria with significant biocontrol activities. The modes of action of several of these strains is currently being investigated.

The Nicotiana benthamiana Hsp-alpha protein (NhHsp-alpha) interacts with the movement protein of the bipartite begomovirus Bean dwarf mosaic virus

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Geminiviruses are plant-infecting single-stranded DNA viruses. Bipartite members (genus Begomovirus) encode a nuclear shuttle (NSP) and movement protein (MP) to facilitate trafficking of viral DNA across nuclear and cell wall boundaries, respectively. A yeast two-hybrid screen was performed to identify Nicotiana benthamiana host proteins that interact with the MP of the begomovirus Bean dwarf mosaic virus (BDMV). One potential MP-interacting protein identified in the screen was a small heat-shock protein (Hsp), NhHsp-alpha. NhHsp-alpha is a plant specific Hsp with homologues in Arabidopsis thaliana and other plant species. There are several subfamily groups of small Hsps in general, some of which function as molecular chaperones. We obtained the full-length NhHsp-alpha gene from N. benthamiana and confirmed that NhHsp-alpha interacts with the BDV MP in vivo by yeast two-hybrid and co-immunoprecipitation assays. Transient expression of a fusion protein in N. benthamiana leaves revealed localization to punctuate structures in the cell membrane and wall. Evidence these were plasmodesmata came from co-localization of RFP-Hsp-alpha with a green fluorescent protein (GFP)-Tobacco mosaic virus (TMV) MP fusion protein. The role of NhHsp-alpha in BDV infection was assessed in plants overexpressing the protein or in which the gene was silenced.

Nematicidal activity of plant essential oils and components from Gaultheria fragrantissima and Zanthoxylum alatum against pine wood nematode

Pine wilt disease, caused by the pine wood nematode, Bursaphelenchus xylophilus, was firstly reported in Busan, a city located in south-eastern coast of Korea. Since then, it has spread to several areas of the peninsula. In this study, we investigated the nematicidal activity of 29 commercial plant essential oils and some of their components which have not been tested before against B. xylophilus. Good nematicidal activity against B. xylophilus was achieved with essential oils of Gaultheria fragrantissima and Zanthoxylum alatum. GC-MS analysis of the corresponding oils led to the identification of 2 and 10 major compounds, respectively. Four compounds such as methyl salicylate, ethyl salicylate, methyl trans-cinnamate and ethyl trans-cinnamate were tested individually for their nematicidal activities against the pine wood nematode. Methyl and ethyl salicylates showed strong nematicidal activity at concentration of 2.0 mg/mL. Concentrations of 1.0 mg/mL as well as lower concentrations showed only minor effects. Another compound, methyl trans-cinnamate, showed 100% activity at concentrations of 0.0625-2.0 mg/mL. In case of ethyl trans-cinnamate, 100% mortality was observed at concentrations of 0.25-2.0 mg/mL. The essential oils and their components described herein merit further study as potential nematicicides against the pine wood nematode.

Co-packaging of genomic RNAs and virion accumulation are controlled by the N-terminus of the Red clover necrotic mosaic virus capsid protein

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Phytopathology 101:S137

The RCNMV genome is composed of two ssRNAs, RNA-1 and RNA-2, that are packaged into two distinct virion populations containing either one copy of each genomic RNA or 4 copies of RNA-2 only. The N-terminal 50 amino acids of the RCNMV CP are rich in basic residues and are essential for RNA binding. Here, we constructed a series of alanine substitution mutations in the N-terminal region of the CP and investigated the biological significance of these basic residues during encapsidation and infection. Our results revealed that triple alanine substitutions for lysine residues at either positions 4, 7, and 8 (R1mt) or 25, 33, 38 (R2mt) affected either the ratio of packaged genomic RNAs or virion accumulation levels, respectively. Mutant R1mt induced more pronounced symptoms than a wild-type virus while the mutant R2mt exhibited wild-type symptoms. Furthermore, we found that mutant R1mt exhibited decreased packaging of RNA-1, but not RNA-2, suggesting that this mutation may interfere with specific recognition of RNA-1 and co-packaging of both genomic RNAs during encapsidation. In the case of mutant R2mt, the mutation did not affect systemic infection and symptom expression but exhibited significantly reduced virion accumulation levels. Taken together, these results suggest that lysine residues at positions 4, 7, and 8 on the N-terminal 10 residues play an important role in specific recognition of RNA-1 and/or the complex of RNA-1 and RNA-2 for co-packaging of both genomic RNAs.

Forest Phytophthora of the World website

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Phytopathology 101:S137

Phytophthora diseases threaten the biodiversity and sustainability of forests globally. Researchers around the world are working to better understand Phytophthora organisms, to recognize newly emerging diseases they cause, and to develop management practices that minimize the spread and severity of disease. Information on forest Phytophthora is scattered, making it difficult for researchers and land managers to stay abreast of recent scientific publications, and educational materials. Our website aims to provide scientific and educational resources to aid in the international understanding and management of forest Phytophthorases. The website includes descriptions of each forest Phytophthora species including morphology, growth characteristics, phylology, host range, ecology, and disease symptoms. Citable articles on individual species will be published as the online journal, Forest Phytophthora. Our website features include educational and management resources; a searchable photo gallery; an easy-to-use synoptic key; a disease finder to identify potential causal organisms based on known management resources; a searchable photo gallery; an easy-to-use synoptic key; a disease finder to identify potential causal organisms based on known hosts, location and symptoms; links to a global mapping function; an illustrated glossary; and key references in a searchable database with easy export options. Access the website at www.ForestPhytophthoras.org.

Historical pathways of introduction for non-indigenous forest pathogens

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Non-indigenous pathogens introduced since the late 1800s have caused sixteen damaging forest epidemics that have reduced or now threaten the diversity and sustainability of U.S. forests. Evidence for the most likely pathway of introduction is provided for these invasive forest pathogens based...
on historical records and knowledge of pathogen biology. Eight pathogens were likely introduced on live plants: Cronartium ribicola, Cryphonectria parasitica, Discota destructiva, Gremmeniella abietina var. abietina, Lachnellula willkomiis, Melampsora larici-populina, Phytophthora lateralis, and P. ramorum. Two insect-vectored pathogens, Ophiostoma ulmi and O. novo-ulmi, were imported on logs, and another, Rafflesia larvaeolaria, was likely introduced on solid wood packaging. The entry pathway could not be determined for five pathogens: Ceratocystis fagacearum, Cryptodiaphoropore populea, Phytophthora cinnamomi, Sirococcus clavigignenti-juglandacearum, and the Venturia saliciperda-Glomerella miyabeana complex. Identification of pathways is critical to preventing new pathogen introductions. Findings emphasize the importance of improved mitigations for pathogenesis on live plants as global plant trade escalates.

**Seasonal variation of Candidatus Liberibacter asiaticus in citrus branches and in vector, Diaphorina citri, in Central Florida sweet orange groves**

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Phytopathology 101:S138

Candidatus Liberibacter asiaticus (Las) causes the insect vectored disease Huanglongbing (HLB) in citrus spp. In Florida, the disease rate has increased two-fold every year since 2005. Significant knowledge gaps exist in Las transmission and epidemiology. Sweet orange groves in central Florida were selected to study seasonal dynamics of Las over a 3-yr period in host and vector (Asian citrus psyllid; ACP). Two to three hundred trees were selected in each grove and 1 leaf/tree was collected randomly every fortnight. Sample collection began in June 2010 with collections ongoing. For qPCR detection of Las, the midrubs of 2–5 leaves were randomly pooled to obtain 30–40 samples/date. An estimated Las prevalence in the branches was calculated from the pools with POoldInRate v3. Simultaneously, ACP collected from the same location were pooled at 1–4 ACP/sample for qPCR. Las prevalence increased in the moderately infected grove from summer to fall (25 to 36%) and decreased in winter (21%), while the highly infected grove had 42, 44, and 59% infection in summer, fall and winter respectively. Las prevalence in ACP also increased from summer to fall in moderately (16 to 43%) and highly (37 to 53%) infected groves. Seasonal dynamics of Las in ACP and citrus followed a similar pattern over summer and fall. If the trends are consistent for other seasons, it is likely that host and vector have similar seasonality, and that could be exploited to manage HLB.

**Using phenotypic markers to identify common beans with two and three rust resistance genes**

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Two new races of the rust pathogen (Uromyces appendiculatus) of common bean (Phaseolus vulgaris) appeared in Michigan and North Dakota in 2007 and 2008, respectively. These races rendered susceptible many previously rust resistant dry bean varieties carrying the widely used Ur-3 rust resistance gene. Furthermore, mutation within the secretin protein gene pilQ resulted in reduced virulence toward fungal host cells. These results support a multifunctional role for T4P in E. enymogenes, including a significant role in pathogenesis of fungal hosts.

**Management of charcoal rot of sweet potato in India**

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Phytopathology 101:S138

Altogether 14 isolates of Macrophomina phaseolina (Tassi) Goid. from different states of India were screened for their sensitivity against carbendazim both in vitro and in vivo. The effect of passage on most carbendazim sensitive isolate was tested by exposing to carbendazim continuously, alternately with captan, zineb and mancozeb and in mixture with them. This was studied both in vitro and in vivo. The most carbendazim resistant isolate (91%) was also resistant to carbendazim under conditions of various agrochemicals for its management. It was observed that carbendazim with captan, zineb, mancozeb, methomyl, endosulfan, monocrotophos, 2, 4-D, excel mera 71, zeepclav 500, griseofulvin, oflaxacin 400, potassium chloride, sodium chloride, manganese chloride, urea, muriate of potash, iron, molybdenum, cobalt, copper and manganese completely inhibited the growth of pathogen both in vitro and in vivo.

**Comparison of pecan scab predictions in Oklahoma using weather inputs from the National Weather Service, the Oklahoma Mesonet, and onsite-monitoring**

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Phytopathology 101:S138

Inputs from networks of weather stations are often used in disease models to assist growers in timing fungicide sprays. A standardized national weather station network does not exist, despite disease epidemics affecting crops across the U.S. The National Weather Service (NWS) has a network of non-standardized stations, which can be used to predict scab (caused by Fusarium effusum) epidemics across the U.S. pecan belt. In Oklahoma, the Mesonet is used for collecting weather inputs used by a pecan scab advisory system. Although couplings (SH; an hour where average T ≥ 21.1°C and average RH ≥ 90%) are accumulated over a 14-d period. In 2010, SH was compared between 15 NWS and Mesonet stations in closest proximity to one another. On-site weather data were collected to determine SH at two sites in 2009 and one site in 2010. Regression analysis and T-tests were used to determine quantitative differences in daily SH for each comparison. Five NWS stations significantly (P > 0.05) over-predicted SH, while six NWS stations significantly (P >0.05) under-predicted SH. Both the NWS and Mesonet significantly under-predicted SH compared to on-site SH at one location in 2009 and 2010. No significant differences were identified in SH measured by the on-site weather station and Mesonet in 2009. While NWS stations can be used to determine SH across the pecan belt, accuracy of SH measurements might be improved through site-specific interpolation of weather data.

**Persistence of the walnut twig beetle in black walnut logs as influenced by chemical and cultural treatments**

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Phytopathology 101:S138

The walnut twig beetle (Pityophthorus juglandis) is the vector of Geosmithia morbida, the cause of thousand cankers disease of black walnut (Juglans nigra). We studied whether clear plastic tapping or applications of bifenthrin, permethrin, and biodiesel to felled logs influenced the persistence of the walnut twig beetle in bark or altered the suitability of logs for future breeding by the insect. Logs from trees with thousand cankers disease were treated and arranged in a randomized complete block design at two Colorado locations. At periodic intervals sections of the logs were removed and placed indoors in insect emergence boxes. Beetle emergence from all logs at the two sites was variable six months after trees were felled; no beetle emergence was recorded.
in a portion of both treated and untreated logs. Nevertheless, only logs treated with bifenthrin were consistently devoid of beetles. Walnut twig beetles emerged from a small proportion of the treated and untreated logs after 18 months and even from some logs in which the beetles previously had not been detected. Geosmithia morbida was isolated from the walnut twig beetle at each sampling date. These results indicate that logs can remain a source for both the beetle and fungus for at least 18 months.

**Witch’s broom phytoplasma infecting Echinacea pallida in Australia**

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Phytopathology 101:S139

Purple coneflower (Echinacea pallida), is commercially cultivated in Australia for its medicinal properties. In 2005, coneflower fields in Tasmania, Australia began exhibiting symptoms typical of phytoplasma infection, including virescence, phylloidy and chlorosis. Surveys of fields in 2011 indicated the presence of symptoms within one field only. Disease distribution in this field was assessed by hierarchical sampling, incorporating 10 spatially referenced transects. Along each transect, 20 individual plants were assessed at 1 m intervals for the proportion of symptomatic flower heads. Overall, incidence of infected plants was estimated at 32%, while the mean percentage of symptomatic flower heads was 12%. Spatial analysis indicated a random distribution of symptoms across the field. Phytoplasma infection was confirmed by DNA sequencing of a 1.2 kb region of the 16S RNA gene, obtained by amplifying total DNA extractions from symptomatic coneflower tissue. Individual sequences shared greater than 99% homology with 'Candidatus Phytoplasma australasiae'. Comparison of virtual restriction fragment profiles from the same genetic region confirmed that the pathogen belonged to the Witch’s Broom (16SrI-D) group of phytoplasmas. All previous reports of phytoplasma infection of coneflower have indicated Aster Yellows (16SrI-C) group phytoplasmas as the causal agents.

**Functional analysis of Asian soybean rust resistance pathways using virus-induced gene silencing**


Phytopathology 101:S139

Asian soybean rust is an aggressive foliar disease caused by the obligate biotrophic fungus Phakopsora pachyrhizi. Outbreaks of the disease have the potential to severely affect overall yields, and all commercially grown elite cultivars of soybean are susceptible to the pathogen. Gernsplaum screening efforts have identified five different genes (Rpp1 - Rpp5) that confer varying levels of resistance to select isolates of P. pachyrhizi. We are using virus-induced gene silencing (VIGS) in an effort to identify and characterize these resistance genes and the gene networks through which they operate. In recent years, VIGS has become an important molecular tool for studying the function of specific plant genes, including those involved in defense. A set of DNA-based VIGS vectors has been developed for use in soybean, and when combined with large scale genomic sequence, transcriptome analysis, gene mapping studies, and metabolomic data, VIGS is a powerful reverse genetic tool to assess functionality. To date, we have screened over 150 soybean genes and have identified several that compromise resistance when silenced. Here we provide an overview of the approach and a summary of genes that contribute to rust resistance.

**Comparing the efficiency of visual scouting, spore trapping systems and a bioindicator for early detection of Erysiphe necator in California vineyards**


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Phytopathology 101:S139

Powdery mildew (PM) caused by Erysiphe necator is the most serious disease of grapevines in California. Ascosporas constitute the primary inoculum in vineyards where chasmothecia have overwintered. Air currents disperse spores, and airborne concentrations are largely a function of the local population of the pathogen. Early detection at low density is a key tactic in the management of the disease. In this work, three different detection methods were compared during two consecutive seasons in five vineyards in multiple locations in California. Rotorod and ionic spore trapping systems, both coupled with quantitative PCR using specific probes and primers, were used to monitor ascospore release and conidia dispersal throughout the season. Efficiency was compared to that of visual/manual scouting and bioindication by a native mycophagous beetle, Psylllobra vigintimaculata, an obligate consumer of E. necator. Initial insect incidence was positively correlated with initial PM incidence. Moreover, the two spore trapping systems were significantly different in terms of detection efficiency.

**Microbial ecology of soils and strawberry roots in non-treated soils that appear to enhance plant growth compared to fumigated soils**

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Soil fumigation using methyl bromide (MB) is used as a pre plant treatment to control a range of pathogens in crop production systems. A strawberry grower MB suppressed strawberry plant growth in one field compared to adjacent non-fumigated areas, an unusual phenomena and opposite of expectations. Planting occurred over 4 weeks after application suggesting chemical toxicity was not a factor. We hypothesized this was a biologically mediated process. Treated (MB) or non-treated (NT) soils were collected in a pairwise-sampling design in the spring at peak harvest. Strawberry leaf dry weights were 10.4 g/plant from MB treated rows and 29.8 g/plant in non-treated rows (P < 0.001). Soil and strawberry roots were tested for the presence or absence of pathogenic and beneficial microbes. Soil microbial numbers were counted using grow-out dilution assays. The strawberry root rot pathogens, Pythium and Fusarium populations were dramatically suppressed to 1175 and 1096 colony forming units per gram of dry soil (cfu/gds) in MB treated soils compared to 3255 and 10,058 cfu/gds in NT soils, respectively (P-value < 0.001). Therefore, MB treatment effectively reduced pathogen populations. Soil bioassays with cross inoculations of soils were conducted to further determine if the NT soil harbored beneficial communities or if MB treatment negatively affected plant growth. Follow-up field studies have been implemented to further clarify the unique phenomena experienced is this particular field site.

**Curtovirus quantification and species differentiation within mixed infections through real-time PCR**

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Phytopathology 101:S139

Curly top is an important viral disease found throughout the United States. Viral infection causes chlorosis, malformation of leaves, stunting, and a reduction in yield. In chile, Beete Severe Curly Top Virus (BSCTV), Beete Mild Curly Top Virus (BMCTV), Beete Curly Top Virus (BCTV), Pepper Curly Top Virus (PeCTV), and Pepper yellow dwarf virus (PeYDV) are the species most commonly found. Current detection methods are qualitative and unable to differentiate among species commonly infecting chile. Utilizing Real-time PCR (Q-PCR), a novel system was developed to quantify and differentiate Curly top species within a mixed infection.

**The occurrence of Cucurbit chlorotic yellows virus disease in Taiwan and evaluation of the virus infected fruit quality and yield**

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Phytopathology 101:S139

Melon (Cucumis melo L.) and watermelon [Citullus lanatus (Thunb.) Matsum & Nakai] are two high-value cucurbit crops and occupy plantation areas of more than 5,000 ha and 13,000 ha in Taiwan, respectively. The Cucurbit chlorotic yellows virus (CCYV), members of the genus Crinivirus, is of the major threats for the cultivation of cucurbit in Taiwan. This virus is whitefly-transmitted crinivirus. From 2009–2011, we survey 538 melon field. Based on the locations, townsships of Yanlin, Chiayi, and Tainan are regarded as southern Taiwan. The melon infected percentage of Cucurbit chlorotic yellows virus (CCYV) was only 0, 6% and 9% in 2009, 2010 and 2011. The disease incidence upward 75% and losses were 32.8%, and decrease the Brix 5.2 and 1.9. Although this virus was first reported to infect cucurbit in Japan in 2009 (2), in Taiwan
in 2010. The Cucurbit chlorotic yellows virus was widespread and often epidemic in cucurbit crops. The cucurbit crops infected by CCYV have been demonstrated reducing yield by 12.4–32.8%.

Generation of broad-spectrum resistance in transgenic tobacco and tomato plants against distinct tospovirus species of different serogroups

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Phytopathology 101:S140


tospoviruses cause severe damages in important crops worldwide. In this investigation, a conserved region containing the RNA polymerase motifs within the L gene of Watermelon silver mottle virus was used to generate sense-translatable, sense-untranslatable, sense-framedhift, antisense and double-stranded constructs for Agrobacterium-mediated transformation of Nicotiana benthamiana plants. A total of 46.7–70.0% transgenic tobacco lines derived from the five constructs, each with 30 lines, showed complete resistance to WSMoV (the type member of WSMoV serogroup), and 35.7–100% plants of them exhibited broad-spectrum resistance against WSMoV and other different tospovirus serogroup species were also noticed. We conclude that using a single nucleotide fragment corresponding to the L gene conserved region triggers broad-spectrum resistance in tobacco and tomato plants against different serogroup tospoviruses at the genus level.

PacC mediated adaptation to alkaline pH is critical for developing infection hyphae in pathogenic plants in Magnaporthe oryzae


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Phytopathology 101:S140


The Strawberry Advisory System: A forecast system for control of anthracnose and Botrytis fruit rots

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Phytopathology 101:S140


Anthracnose fruit rot (AFR), caused by Colletotrichum acutatum, and Botrytis fruit rot (BFR), caused by Botrytis cinerea, are the main diseases affecting strawberries in Florida. Fungicides are applied on a weekly schedule throughout the season to control these diseases. Calendar-based control programs were compared to applications based on previously published models that related disease incidence to weather variables. These models utilized leaf wetness and temperature during the wet period to predict disease outbreaks. Different thresholds for predicted AFR and BFR incidence were evaluated to trigger fungicide applications. Field trials were conducted for three seasons on two cultivars. The most effective model-based treatments reduced the number of sprays by about 50% without affecting disease control or yield. Selected models and thresholds were used to develop a web-based tool to advise growers of the current disease risk level and the need for fungicide application. The web-based forecasting system, named the Strawberry Advisory System (SAS), was implemented on the AgroClimate website (http://agroclimate.org/tools/strawberry/). Users can also be provided with warnings of the need for fungicide applications via email and/or text messages. In preliminary grower trials, the SAS has been successful in eliminating many unnecessary fungicide applications and has proven user-friendly.

Multiple gene family analysis reveals M. oryzae-associated to native Myrtaceae in Chile

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Phytopathology 101:S140


Multiple gene family analysis reveals M. oryzae-associated to native Myrtaceae in Chile

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Phytopathology 101:S140


M. oryzae is known to be specific to Eucalyptus worldwide. In Uruguay, a relatively large number of Mycosphaerella species are found on Eucalyptus but their occurrence on native Myrtaceae is unknown. Due to the close relationship between introduced Eucalyptus species and native Myrtaceae to Uruguay, the aim of this study was to identify Mycosphaerella species associated with leaf diseases on native Myrtaceae, and to determine their relationship with those affecting Eucalyptus plantations. Several surveys were conducted in native forests throughout the country. Diseased leaves were collected from native host species. Following fungal isolation, cultures were identified based on morphology and comparisons of partial DNA sequences for the ITS, EF-1α and Actin genes. Results revealed the occurrence of Mycosphaerella aurantia, M. heimi, M. yunnanensis and Pseudocercospora norchii, all known to be Eucalyptus pathogens. These results not only suggest the epidemiological importance of native Myrtaceae trees as hosts of exotic Eucalyptus diseases but also raise serious concerns about the movement of pathogens from Eucalyptus plantations to native trees and vice versa. Since most of these species occur on Eucalyptus in countries other than Uruguay, it seems likely that they were introduced into the country and have adapted to infect native Myrtaceae; however, further investigations are needed to test this hypothesis.

Virulence and molecular genotyping studies of Sporisorium reilianum isolates in sorghum

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Phytopathology 101:S140


Head smut, caused by Sporisorium reilianum, has been reported with increasing frequency in the grain sorghum growing regions of Texas. To analyze changes in pathogen virulence, four inoculation techniques were examined: seed and teliospore mixture, seed coating, media placement, and syringe injection. Of the four, syringe injection was determined to be the most effective. Inoculations of sorghum host differentials BTx643, BTx7078, BTx635, SC70-6-17 (TAM2571), SA281 (Early Hegari), and TX414 showed 23 of 32 Texas isolates were race 4. Two isolates from College Station, Texas, were classified as race 1, but no race 2 or 3 isolates were found. New virulent races 5 and 6 were identified among isolates from south Texas. Using 16 AFLP primer combinations, genetic diversity was assessed in DNA samples from 49 S. reilianum isolates, including 44 sorghum isolates from Texas, U.S.A., two from Uganda, and one from Mali; and two maize isolates from Mexico. Single-base extension analysis with EcoRI and MspI primers in the selective amplification increased the number of informative polymorphic bands. High genetic dissimilarity (50%) was observed between isolates originating from maize and those originating from sorghum. The resultant dendrogram, made using cluster analysis, grouped the Texas S. reilianum isolates into four small clusters with >82% similarity. Other than for two race 6 isolates from Westaco, Texas, no evidence for geographical or other restrictions on gene flow was evident.

Genetic diversity and pathotype determination of Colletotrichum sublineolum isolated causing anthracnose disease in sorghum

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Phytopathology 101:S140
Effects of seedborne and overwintering inoculum on ray blight severity in pyrethrum

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Phytopathology 101:S141

Ray blight, caused by *Stagonosporopsis liguicola* var. *inoxidabilis* is an important disease affecting pyrethrum in Australia. Previous studies demonstrated the pathogen can be found within and on seed, although the contribution of this inoculum to epidemic severity is relatively unknown. Logistic regression models were constructed using defoliation severity data collected from 72 commercial fields over nine years. Models based on overwintering inoculum, expressed as the isolation frequency of the pathogen during autumn and winter, or seed contamination incidence and autumn-winter temperature and rain variables were highly predictive of ray blight epidemics. Path analysis was used to model the direct and indirect factors of weather variables and inoculum factors on disease intensity. This analysis indicated that seedborne inoculum of *S. liguicola* var. *inoxidabilis* contributed indirectly to defoliation severity through directly increasing pathogen overwintering frequency. Autumn and winter weather variables had indirect effects on defoliation severity. These findings suggest that losses from ray blight may be minimized through the use of clean seed and/or reducing overwintering inoculum. This could be achieved by growing seed in drier, isolated locations, in combination with other inputs to minimize the probability of disease development.

Characterization of orthologs of Ax21 and two, two-component regulatory systems, phoPQ and coRS, in *Xylella fastidiosa*

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Phytopathology 101:S141

*Xylella fastidiosa* (Xf) is a gram-negative, xylem-limited plant pathogenic bacterium and the causal agent of Pierce’s Disease of grapevine. Biofilms play a key role in early colonization and pathogenicity of Xf by providing a protected niche and enhanced survival in the xylem. In Xf and *Xanthomonas oryzae* pv. *oryzae* (Xoo), biofilm formation is induced by density-dependent gene expression mediated by diffusible signal factors (DSF). Recently, Ax21 was identified as a DSF in Xoo that interacts with two two-component regulatory systems: *rasBH* and *phoPQ*. Orthologs of Ax21, *rasBH*, and *phoPQ* were identified in the Xf genome and deletion knockout mutants were constructed to investigate the functional role of these orthologs in Xf. Using a biosensor assay, we determined Xf does not produce active Ax21, which is likely due to the lack of a sulfation system for Ax21 in Xf. Furthermore, a gene knockout in *Ax21* had no significant impact on biofilm formation but resulted in a 23% reduction in cell aggregation and a 29% reduction in population density after completion of the log phase growth. Knockout strains of Xf deficient in production of phoP and phoQ resulted in a 42% and 47% reduction in biofilm formation, respectively, and a 42% and 36% reduction in cell aggregation, respectively. Our preliminary results indicate that Ax21 and phoPQ play a role in density dependent gene expression during early colonization and infection.

Uptake and translocation of Penthiopyrad fungicide in wheat leaves and correlation to fungal control of key foliar diseases

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Phytopathology 101:S141

Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of a wide range of fungal diseases of cereals and specialty crops. A combination of bioassays, LC/MS, and autoradiographic analyses was used to characterize the degree and rates of uptake and movement into wheat leaves of penthiopyrad in a commercial EC formulation. Penthiopyrad was applied at a field rate using in 10⁻³ L droplets to an area near the base of the leaf. Immediately after treatment most penthiopyrad remained on the leaf surface. Within 28 hours sufficient compound had penetrated and translocated to give efficacious concentrations throughout the leaf. Concentrations sufficient for disease control were maintained throughout 21 days as demonstrated by bioassay results showing both curative and preventive control of wheat leaf rust (*Puccinia triticina*) and leaf blotch (*Septoria tritici*) in untreated zones.

Host modification of *Penicillium solitum* during postharvest decay of apple fruit


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Phytopathology 101:S141

Penicillium *solitum* and *P. expansum* are closely related postharvest fungal pathogens, causing blue mold on pome fruits in storage. *P. expansum* is a more aggressive pathogen; however, the mechanisms governing disparities in fungal virulence are unknown. Modulating the pH of the host environment has been suggested as an important factor that contributes to fungal virulence. *P. expansum* has been shown to modulate its environment via secretion of organic acids into the host tissue with concomitant ammonia uptake during decay development. To determine if the less aggressive nature of *P. solitum* is due to its inability to adequately modify its host environment, we examined the aggressiveness of *P. solitum* on two different apple cultivars, ‘Golden Delicious’ and ‘Winesap’. Wounded apples were inoculated with a *P. solitum* conidial suspension (1 x 10⁹) and evaluated 3, 7, 14 and 21 days after inoculation. Lesion diameter, decay depth, pH, ammonia concentration, and organic acid production in the decayed and healthy tissue were evaluated. On both cultivars, at each time point, *P. solitum* caused similar lesion size and reduced pH inside the lesion, compared to surrounding non-decayed tissue. The observation that *P. solitum* is likely due to the lack of a sulfation system for Ax21 in Xf, resulting in a 23% reduction in cell aggregation and a 29% reduction in population density after completion of the log phase growth. Knockout strains of Xf deficient in production of phoP and phoQ resulted in a 42% and 47% reduction in biofilm formation, respectively, and a 42% and 36% reduction in cell aggregation, respectively. Our preliminary results indicate that Ax21 and phoPQ play a role in density dependent gene expression during early colonization and infection.

Susceptibility of select U.S. winter wheat cultivars to wheat blast (*Magnaporthe oryzae*)

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Phytopathology 101:S141

Wheat blast, caused by a pathotype of *Magnaporthe oryzae*, is an emerging disease in South America. Countries reporting the disease are Brazil, Bolivia, Paraguay and Argentina. Field losses of 30 to 100% have been observed under favorable conditions. The establishment potential of wheat blast in other regions of the world has not been determined, but the spread of this seed transmissible disease is likely. In anticipation of its arrival in the U.S., studies were initiated to assess U.S. wheat cultivars for disease resistance. Reported here are preliminary results of a biological safety level-3 greenhouse screening of 200 U.S. winter wheat cultivars for head blast resistance using a single Brazilian isolate (T-25). For each cultivar, 7 to 20 spikes at growth stage 50 were spray-inoculated with a *M. oryzae* conidia suspension at a rate of 1 x 10⁶ conidia per ml applied until run-off. Individual spikes were enclosed in small a plastic bag to maintain high humidity for 24 h at 23-25°C. After 21 days, spikes were evaluated based in the number of infected florets per spike. Cultivars with less than 10% infection were tested. Test results showed a broad range of susceptibility. Soft red winter cultivars ARS05-00443, GA00067-8E35 and GA0111493-8E18 averaged less than 4% infection, while hard red wheats, KS0603A-57-1, Jackpot, and CO050173 averaged less that 10%. Identification of resistant germplasm will be essential for a U.S. disease recovery plan.
Uptake, transport, and fungicidal efficacy of Penthiopyrad fungicide in wheat resulting in protection of treated and untreated foliage

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Phytopathology 101:S142

Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of a wide range of fungal diseases of cereals and specialty crops. The objectives of this project were to analytically characterize the uptake and movement as well as the persistence of penthiopyrad in a commercial EC formulation applied to field-grown winter wheat at GS32 timing. It was observed that the flag-leaf is fully expanded. Three hours after field application, approximately 50% of the active ingredient was located inside the fully expanded F-2 and the partially expanded F-1 leaves. Five days after application, significant decreases of penthiopyrad were observed in the surface residues of the fully expanded F-2 and in both surface and internal concentrations of the partially expanded F-2. By 14 days after application, concentrations of penthiopyrad in the F-2 and F-1 had not changed significantly since the last sample. Penthiopyrad was detected in the now fully emerged flag-leaf showing the highest concentrations. A subsequent bioassay on greenhouse-grown spring wheat demonstrated that when applied at the GS32 timing when the flag-leaf had not emerged, sufficient active ingredient was transported to the flag-leaf to protect it against leaf blotch (Septoria tritici).

Fungicidal efficacy and partitioning of Penthiopyrad in apple leaves in relation to application rate

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Phytopathology 101:S142

Penthiopyrad is a new succinate dehydrogenase inhibitor (SDHI) fungicide discovered and owned by Mitsui Chemicals, Inc. The active ingredient Penthiopyrad is being co-developed by E. I. du Pont de Nemours and Company for control of fungal diseases in various crops. Our objectives were to determine the partitioning of penthiopyrad applied in a commercial SC formulation between the surface, the cuticle, and inside the apple leaves as affected by application rate, as well as to determine the correlation of partitioning with preventive and curative fungicidal efficacy against apple scab (Venturia inaequalis). We used a 3-compartment partition model based on extraction solvent to evaluate penthiopyrad deposits i) on the leaf surface, ii) associated with the waxy cuticle on the exterior of the leaf, and iii) inside the leaf that was not extracted in previous steps. After application of formulated penthiopyrad to apples, the active ingredient was detected in all three leaf compartments. The relative partitioning of penthiopyrad was depended on the application rate as well as time of sampling after application. At rates similar to field use rates and independent of sampling time, most of the active ingredient was located in the cuticle with lower amounts on the leaf surface and inside the leaf. Both preventive and curative fungicidal activities were directly correlated with increasing application rates of penthiopyrad as well as increasing concentrations of penthiopyrad in the cuticle.

Identification of Fusarium oxysporum f. sp. lycopersici and Fusarium oxysporum f. sp. radicis lycopersici using specific primer

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Phytopathology 101:S142

Fusarium oxysporum f. sp. lycopersici (FOL) and Fusarium oxysporum f. sp. radicis lycopersici (FORL) cause considerable damages in greenhouse. FOL is only pathogenic on tomato and FORL is pathogenic on other hosts from Cucurbitaceae family in addition to tomato. In this study, 35 fungal isolates from the border of healthy and infected tissues of tomato vessels with wilt symptoms were identified as Fusarium oxysporum. Pathogenicity of isolates was investigated on tomato and cucumber on seedling stage with conidial suspension inoculation (106 spores/ml). 13 isolates caused wilt in cucumber in addition to tomato. Study of growth rate of isolates on PDA in 18°C and 27°C temperatures showed that 13 isolates which were pathogenic on cucumber have more growth in 18°C. To confirm the above results, DNA of isolates were extracted and reproduced by using Uni and Sprl specific primers. All isolates with Uni primer made a 672 bp band. In addition, 13 isolates with Sprl primer made a 947 bp band. Therefore, the latter isolates were determined as FORL and the other 22 isolates were determined as FOL. In this way, PCR molecular method confirmed the accuracy of above results with more precision.

Identification of a novel fruiting structure produced by Aspergillus niger and A. carbonarius in grape berries affected by sour rot

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Phytopathology 101:S142

Aspergillus spp. infections of grapes can lead to sour rot. Aspergillus enters the berries through wounds caused by birds, insects, or other mechanical damage at veraison or later. Typical sour rot symptoms include soft rot of berry tissue followed by colonization by other fungi, acetobacter and yeasts. A novel sporulation structure caused by Aspergillus spp. has been observed in berries affected by sour rot. Sporulation occurs at the formation of a cavern-like structure of soft fungal tissue growing inside the berry. Isolates of A. niger and A. carbonarius were used to conduct field and laboratory experiments to monitor the formation and development of the fruiting structure over time. Identification of the fruiting structure was done by means of histological studies with GMS and H&E stains to better understand the developmental stages of both Aspergillus spp. on the table grape Red Globe. The effect of temperature was also studied. Individual berries were wound inoculated and incubated at different temperatures. It was observed that structures of two kinds were formed under dry conditions: a “cavern”- type structure that assumed a more tubular shape, and a thinner fungal tissue forming around the seeds of Red Globe grapes. Histological studies showed that conidia-bearing structures start forming around 16 days post inoculation on the fungal tissue closest to the seeds.

Tropical race 4: Current and future impact on export and subsistence banana production

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Norman Simmonds classified fusarium wilt of banana, caused by F. oxysporum f. sp. cubense (FOC), as one of the most destructive plant diseases. Susceptibility to race 1 of FOC doomed ‘Gros Michel,’ the banana cultivar on which the first export trades were based; it was replaced by race 1-resistant Cavendish clones by the 1960s. Currently, the tropical American trades depend on the Cavendish subgroup. A new variant of FOC, tropical race 4 (TR4), has recently decimated Cavendish monocultures in southeastern Asia. We summarize the threat that TR4 poses to global production. TR4 would affect 85% of total production were it disseminated to other important banana-growing regions. In the Americas, a hemispheric plan has been drafted to address courses of action for before and after TR4 would arrive in the region; it has been discussed with regional quarantine authorities, growers and scientists, and is used to focus activities that surround the issue and inform a research strategy. For example, a contingency plan was developed by the USDA Decades of research on race 1 resulted in no highly effective management options for infested soils other than the use of resistant genotypes. Thus, as expected, biological and cultural measures have proven to be ineffective long-term measures for managing the TR4 problem. We discuss the conventional and nonconventional improvement of banana with TR4 resistance, the recent development and application of a TR4 diagnostic, and future research objectives.

Genetic variability of Colorado Cherry rasp leaf virus

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Cherry rasp leaf virus (CRLV) is one of the most common viral pathogens of tree fruits in Colorado. Infected trees show different symptoms including mild to severe emotions in orchards. To assess the incidence and genetic variability of CRLV, 267 samples were randomly collected from stone and pome fruit orchards in western Colorado from June-October 2010. The virus was detected from 80 cherry and apple samples by RT-PCR using virus-specific primers. A DNA fragment of 452-bp from a conserved RNA dependent RNA polymerase region was cloned and sequenced from 36 samples. Comparisons of the sequence of the 36 isolates with the sequence of the National Center for Biotechnology Information (NCBI) database from the GenBank database show that the Colorado isolates share 92–100% (majority 96–100%) identities with each other and 87–100% with the three known isolates. Phylogenetic analysis of the deduced aa sequences indicates low genetic variation among the Colorado isolates, with a majority (35 of 36) grouped in a major cluster. There are three subclusters within the major cluster, with two of them containing isolates from different host species and orchards, indicating a lack of correlation among variants, hosts and orchards. A variant from sour cherry was the most distinct among the Colorado isolates.
Incidence of multiple viruses in western Colorado cherry orchards
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A limited survey for viruses in commercial cherry orchards in western Colorado in 2008 and 2009 suggested there may be a correlation between leaf symptoms and viral infection. Thus, further investigations were performed in 2010. Leaf and fruit samples from 116 trees of various sweet and sour cherry cultivars were collected from 25 orchards. Total nucleic acids were extracted and tested for viruses (RT-PCR) and viroids (dot blot hybridization) to correlate the severity of leaf enation symptoms, typically associated with Cherry rasp leaf virus, with the presence of other viruses or viroids. Eight viruses including CRLV, Cherry virus A (CVA), Cherry green ring mosaic virus, Cherry necrotic russet mottle virus, Plum bark necrosis stem pitting associated virus, Prune dwarf virus, Prunus necrotic ringspot virus and Tomato ringspot virus were found in various combinations in these trees. No viroids (Peach latent mosaic viroid and Hop stunt viroid) were detected. At least one virus was detected in 94% of the samples, with CRLV (62%) and CVA (53%) being the most common infections. Two or more viruses were present in 60% of the samples, and combinations of up to 7 viruses were detected in a given tree. The incidence of multiple infections did not correlate with symptom types, varieties or locations. Although trees with leaf enations were infected with CRLV, asymptomatic trees were also found to contain CRLV.

Efficacy of bio-fumigation and soil solarization on soil-borne onion pathogens
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Phytopathology 101:S143
Bio-fumigation can manage many soil-borne pathogens, but its efficacy is not known against Fusarium Basal rot and pink root diseases of onion. Studies were conducted to evaluate the efficacy of soil solarization, biofumigation, and their combination on these diseases within the context of existing growers cropping systems. The field studies used a replicated split-split plot completely randomized block design; these were followed by greenhouse experiments. Treatments included mustard vs no mustard as main plots; canola meal cake, chicken manure and control as sub plots; and plastic mulch vs no mulch as sub-sub-plot treatments. Mustard growth after sweet corn harvest in 2008 was incorporated into the soil along with chicken manure and canola meal cake in Sept. and temperature sensors were inserted at 15 cm depth to monitor soil temperature. The soil was covered from Sept. 19 to Oct. 30 with four mm thick transparent plastic sheets in designated plots that were made air tight from all sides. Onion was grown in the next season and Fusarium basal rot and Pink Root incidence was measured toward the end of the growing season. Plastic mulch increased soil temperature, and soil amendments facilitated the process. Mustard, canola meal cake, chicken manure, and their combination did not affect Fusarium basal rot incidence; only chicken manure reduced the Pink Root incidence. A combination of mustard, chicken manure and soil solarization was more effective than each factor alone.

Impact and characterization of ‘black shadow’ on highbush blueberry
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There is an increasing occurrence of a disease that we call ‘black shadow’ on highbush blueberry (Vaccinium corymbosum) stems and buds. The disease is characterized by a blackening of the 1–2 year old shoots and the developing buds. Symptoms become apparent in the late summer and fall and are readily visible in the spring before the plants emerge from dormancy. The blackening is due to the growth of fungi in or on the plant cuticle. To characterize the problem, we isolated the causal agent(s). Small sections of affected bark were plated on PDA. At least three different types of fungi were isolated, which were similar in size to many blueberry and choke speck of apple. However, the disease is distinct from a similar condition known as sooty mold which develops epiphytically on plant exudates and the sugary excretions of some insects. To quantify the impact, shoots were collected from affected bushes and evaluated for percent ‘black shadow’ coverage, length (current year growth), and number of flower buds. Preliminary results suggest that when black shadow covers greater than 90% of the stem surface, the inflorescence buds are reduced in size and number. Control of the disease is best accomplished with application of lime sulfur in the fall. Work is ongoing to confirm the causal agent(s).

Potential of Fourier transform infra-red (FTIR) spectroscopy for differentiation of phytopathogens
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Phytopathogenic fungi are of the most common causes of plant disease. Distinguishing among different strains or isolates of a certain fungus species is difficult, and time consuming using common biological means. In this study we suggest a quick and low cost technique. IR spectra are well known for their sensitivity to composition and three dimensional structures of biomolecules. They allow measuring complex molecular vibrational modes of functional groups. FTIR spectroscopy has become a widely used analytical tool in the biomedical sciences for characterization of a variety of specimens, e.g., microorganisms, body fluids and tissues. In this study we used FTIR attached with attenuated total reflection (ATR) to examine fungi. Using this technique one can get information of the fungus chemical structure which is represented in its mid-infrared absorption spectrum. Comparing absorption spectra of various fungi genera shows significant differences among them. Using cluster analysis techniques, we differentiated between different genera with 100% success. Further examination of several isolates of the same species (Fusarium oxysporum) yielded more subtle differences among the absorption spectra. In order to distinguish among the isolates, we used advanced mathematical and statistical methods (PCA and LDA respectively) on the obtained spectra. That resulted in good distinction (90%) among the isolates, which is, to our knowledge, an unprecedented result in this field.

Molecular diversity of viruses in vegetable crops from farmers’ fields in South and Southeast Asia
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Phytopathology 101:S143
Virus diseases are affecting the production of vegetables by farmers in South and Southeast Asia. The IPM-CRSP of the USAID has funded a project to implement ecologically-based IPM packages for key virus diseases and/or virus-vector complexes. For this purpose, we have collected samples suspected for virus infections, based on visual symptoms, from a variety of vegetable crops (tomato, chili peppers, okra, bitter gourd, bottle gourd, cucumber, pumpkin and ridge gourd) in farmers’ fields from India, Bangladesh, Nepal, Cambodia, and Indonesia. These samples were imprinted on FTA® cards or nitrocellulose (NC) membranes in the field, air dried and
shipped to Washington State University. Total nucleic acids recovered from FTA cards and NC membranes were tested by PCR and RT-PCR using group- and species-specific primers for the detection of a range of viruses. The DNA fragments amplified from these assays were cloned and nucleotide sequence determined. A comparison of these sequences with corresponding sequences available in GenBank revealed the presence of distinct virus species belonging to the genera Begomovirus, Potyvirus, Tosopovirus and Ciclovirus. These results provided a foundation for a better understanding of the spectrum of viruses in vegetable crops across the regions and the development of field-based assays for their monitoring in multi-location varietal evaluations and IPM trials in host countries.

**Rapid and real-time detection of grapevine leafroll associated viruses in grapevines and insect vectors**

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Grapevine leafroll disease (GLRD) produces distinct symptoms in red- and white-fruited wine grape cultivars of *Vitis vinifera*. GLRD symptoms are sometimes mimicked by nutritional disorders, mechanical damage during viticultural operations and injury due to other abiotic factors, especially in red-fruited wine grape cultivars. Previous studies have shown that six grapevine leafroll-associated viruses (GLRaVs; GLRaV-1, -2, -3, -4, -5, and -9) are present in GLRD-affected grapevines in Washington vineyards. Due to low concentration and uneven distribution of viruses in grapevines and variation in disease symptoms, we developed reverse transcription-quantitative real time polymerase chain reaction (RT-qPCR) assay for the detection of GLRaV-2 and GLRaV-3 in grapevines infected with GLRD. For this purpose, we designed primers to amplify a 120 base pair fragments specific to the replicase gene module of GLRaV-2 and GLRaV-3 and optimized conditions for their amplification in grapevine samples and insect vectors. Using this method, we were able to estimate virus load in a given sample. The RT-qPCR provided higher sensitivity for the detection of the two GLRaVs in grapevines and vectors and allowed rapid discrimination of these viruses in mixed infections. This assay is being used in monitoring the spread of GLRD and discriminating GLRD from 'symptoms' due to nutritional and other abiotic factors.

**Identifying quantitative trait loci (QTL) for resistance to Fusarium crown rot (Fusarium pseudogibberineum) in two spring wheat populations**

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Fusarium crown rot (FCR), caused by *F. pseudogibberineum* and *F. culmorum*, reduces wheat yields in the Pacific Northwest (PNW) of the U.S. by as much as 35%. Currently there is no consistent durable resistance to FCR in PNW wheat cultivars. Significant QTL for crown rot resistance have been documented on chromosomes 1A, 1D, 2B, 3B, and 4B from resistant Australian cultivars. The objective was to identify major QTL for Fusarium crown rot resistance in the Australian spring wheat cultivar ‘Sunco’ to facilitate PNW breeding efforts. Two mapping populations consisting of 151 F5:F6 and 219 F6:F7 recombinant inbred lines (RIL) were derived from crosses between Sunco (partially resistant) by Otis (susceptible) and Sunco by Australian spring wheat cultivar ‘Sunco’ to facilitate PNW breeding efforts. Two mapping populations consisting of 151 F5:F6 and 219 F6:F7 recombinant inbred lines (RIL) were derived from crosses between Sunco (partially resistant) by Otis (susceptible) and Sunco by Macoun (susceptible), respectively. Plants were inoculated with *F. pseudogibberineum* isolate (006-13) in growth room (seedling), outdoor nursery (adult) and field (adult) assays. Stem base crown tissues of seedling and adult plants were collected for disease severity on 0 to 10 scale during 2008–2009 and 2010. Five significant QTL were identified on chromosomes 3B, 4B, 4A, and 7A with LOD scores ranging from 3.0 to 23. The most significant QTL was located on chromosome 3BL and inherited from Sunco with maximum LOD scores of 23 and 10 explaining 28% and 23% of the variation, respectively for each population. This is the first report of this unique 3BL QTL for resistance to Fusarium crown rot inherited from Sunco.

**Spatial distribution of soybean cyst nematode in research plots**

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Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe (Tylenchida: Heteroridae), is the most important pathogen of soybean (*Glycine max* (L.) Merr.), in the United States. The spatial distribution of SCN in ten naturally infested sites in the Red River Valley of North Dakota was examined during 2006–2009. These sites, which ranged from 557 to 975 m², had been used as disease nurseries to screen soybean cultivars. SCN populations varied among plots from undetected to 25,000 eggs/100 cc soil, and in some sites the differences in egg densities observed between adjacent plots were as high as 6-fold. Lloyd’s index of patchiness, which ranged from 1.09 to 3.34, suggested an aggregated distribution in nine of the ten sites evaluated. Egg densities were grouped in classes using two categorical scales based on the effect such populations have on soybean yield. Such data was used to estimate the effect of the spatial variability of egg populations on plot size and to estimate the minimum number of plots required to compare cultivars. In three of the sites, no minimal plot size could be determined due to the spatial distribution of SCN. For some sites, the use of categorical scales compared to using egg numbers, reduced the size and number of plots. The spatial distribution of SCN eggs in research plots can be a critical factor affecting outcomes of field experiments.

**Survival and natural biological control of Sclerotinia sclerotiorum in alfalfa seed production**

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White mold caused by *Sclerotinia sclerotiorum* can be a major issue for alfalfa seed growers under cool wet weather conditions following row closure. After harvest in August, alfalfa seed growers in Touchet, WA manage stubble by burning it in February to March of the following year. Burning alfalfa stubble is practiced to help manage weeds, insects and white mold, however, pressure to limit or not permit burning is a current issue due to environmental concerns. The present study determined that the total number of sclerotia in the soil of burned field plots was either less (41%) or significantly less (71%, P = 0.015) than non-burned control plots in 2009 and 2010, respectively. In addition, survival of sclerotia in burned verses non-burned plots was either less (9.4%) or significantly less (10.7%, P = 0.021) than non-burned field plots in 2009 and 2010, respectively. The percentage of sclerotia colonized by *Fusarium acuminatum* collected in plots that were burned (23.8% in 2009, 24.2% in 2010) was significantly greater than in plots where stubble was not-treated, mowed or tilled in 2009 (P = 0.0533). *F. acuminatum* and *Ulocladium atrum* were identified as the two dominant natural colonizers of sclerotia in field soil. These results support that field burning of alfalfa stubble is an important IPM practice for the management of white mold in seed production.

**Biological control of Silvery threadmoss (Bryum argenteum) a weed problem of golf course putting greens and nursery crops**

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Silvery threadmoss (Bryum argenteum) has become an increasingly problematic weed of golf courses, particularly since the loss of mercury and other post chemical based programs, which controlled moss. A decrease in mowing height requiring increased passes of equipment over the green, decreased nutrient inputs, and an open turf canopy contributes to moss encroachment on putting greens. The only commercial herbicide labeled for moss control is carfentrazone which does not completely eradicate moss, so sequential applications are required once moss recovers. Aside from turf, quality and yield, branch/tree dieback, and orchard devastation, Xap is endemic in the U.S.A., NZ and is a quarantine regulated pathogen in Europe.
and elsewhere. Almost nothing is known about the genetics of Xap compared to other xanthomonads. We have sequenced the complete genome of a genotypic-representative Xap strain from Europe (Italy, CFBP 5530). This is the first complete genome sequence for this species. Paired-end 454-pyrosequencing and primer walking on a fosmid library gave 3 contigs. The chromosome is 4.85 Mb with 65.6% GC ratio and 3912 predicted CDS. Xap has a unique 41.2 kb plasmid with a 62.3% GC ratio and 45 CDS. Automatic annotation using several sequenced Xanthomonas genomes as templates, and partial manual annotation, identified a suite of 21 type III secretion system effectors, iron acquisition, and other putative virulence/ecological fitness determinants, many apparently unique to X. arboricola. EDGAR comparisons against X. axonopodis pv. citri strain 306 and X. campestris pv. campestris LMG8004 indicated a pangenome of 2786 CDS and 848 singletons in Xap. Applied genomics has identified over 90 VNTR, several of which are currently being used for biodiversity analysis of Xap and related subspecies, and design of improved diagnostics.

MALDI-TOF mass spectrometry: Applications for rapid bacterial identification and phylogenetic analysis


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Rapid reliable identification of pathogenic bacteria is critical for effective implementation control measures, and is typically required at subspecies level. We have developed whole-cell matrix assisted laser desorption ionisation – time of flight mass spectrometry (MALDI-TOF MS) as a high-throughput, rapid and cheap alternative for routine diagnostics. MALDI-TOF MS is based on discriminatory peptide mass fingerprints cross-referenced in a comprehensive database. Commercial databases are largely limited to clinical bacteria, and we are filling this gap with super-spectra fingerprints for phytopathogenic bacterial groups (Xanthomonas, Agrobacterium, Erwinia, Dickeya, Pantoea). Sample preparation, analytical and statistical methods have been optimized to deliver robust taxa discrimination at the species and in many cases subspecies level. Further application for rapid phylogenetic analysis has been suggested as a reliable alternative to sequence-based approaches delivering comparable phylogenetic resolution. MALDI-TOF MS methods, applications, comparison with standard identification and phylogenetic approaches, and potential as an emerging tool in phytobiology will be discussed.

Application of bioinformatics to study type III effector signals

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Type III effectors are secreted and translocated into the host cell via a specialized type III secretion system. The process of translocation of effectors is highly regulated. Effectors are believed to contain secretion and translocation signals in the N-terminal region that direct them through the secretion apparatus. Secretion and translocation signals have not been well characterized in xanthomonads. Although a few Xanthomonas effectors contain Pseudomonas effector-like features in the N-terminal region, the majority of the amino acid bias features do not strictly follow for Xanthomonas effectors, indicating the need to develop separate predictive rules and programs for identifying signal features in type III effectors. We have developed position-specific scoring matrices (PSSM) for the motifs identified based on amino acid biases in N-terminal region of Xanthomonas. These PSSMs can be used for screening for the candidate type III effectors from the sequenced genomes and upcoming draft genomes of xanthomonads. Calibration and validation of the PSSMs were carried using already sequenced Xanthomonas euvesicatoria Xcv str. 85-10 genome, from which type III effector repertoire has been well characterized. These matrices were searched for the candidate effectors within bacterial spot xanthomonads. Candidate effectors were tested for translocation using in-planta avrBs2 reporter gene assay. We have identified a few novel effectors from bacterial spot xanthomonads using this method.

Epidemiological studies on Blackberry chlorotic ringspot virus

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Blackberry yellow vein (BYV) has emerged as an important disease in all the blackberry growing areas of southeastern United States, with new viruses associated with the disease discovered continually. Blackberry chlorotic ringspot virus (BCRV), a recently identified irivarirus, has frequently been detected in diseased samples. Other than blackberry, the virus also infects rose and raspberry making its survival more efficient. Apart from the apparent increase in abundance of BCRV in blackberry and rose, there is no information on the epidemiology of this virus. The objective of this study is to acquire information on different aspects of the virus epidemiology including identification of initial sources of infection, alternate hosts and virus transmission. Several isolates of the virus infecting cultivated and wild-blackberry, raspberry and rose were collected from several states. The complete RNA 3 of the virus was amplified, cloned, and subjected to sequence analysis; there is no information on isolate variability. Alternate host identification was performed by testing plant species present in areas with high BCRV incidence. BCRV was grafted onto herbaceous hosts and seed transmission evaluated an efficient transmission mode of irivarirus. Rosa multiflora seeds, naturally infected with BCRV, were also collected and tested for seed transmission. The results of this study clarify factors contributing to the epidemiology of BCRV by identifying the virus variability, alternate hosts and seed transmissibility.

Proteomic and biochemical analysis of heat shock responses in Trichoderma species

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Trichoderma strains are the best studied bioagents of plant pathogens and are successfully employed as biopesticides and biofertilizers. Biocontrol activity of these fungi is significantly affected by soil temperature. Identification of thermotolerant strains of Trichoderma would aid in their successful application in arid and semi-arid regions of agriculture. Trichoderma strains collected from varied agro climatic zones of India were screened for high temperature tolerance by exposing them to 48°C, 50°C and 52°C for 1 hr, 2 hr and 4 hr respectively. Four Trichoderma strains viz., T33, T37316, T653 and T797 with lethal temperature 50 (LT50) of 44–50°C were identified as tolerant to high temperature stress and these strains have good biocontrol potential against root rot diseases caused by Macrophomina phaseolina and Sclerotium rolfsii. These isolates accumulated >25% of trehalose and mannose during heat stress compared to the susceptible strains. Raffinose concentration increased gradually with increased duration of heat stress in all isolates compared to controls and thus its role in thermotolerance is not clear. Cycling decrease and increase of protein concentration was observed in thermotolerant isolates during heat stress at 48°C, 50°C and 52°C for 1 hr and 2D-PAGE analysis revealed accumulation of high molecular weight acidic proteins. Identification of these proteins by MALDI-TOF and their role in thermotolerance are discussed in this paper.

Characterization of bacteriophages PT21 and UASP infecting Ralstonia solanacearum: A potential bio-control agent

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Bacteriophage as biocontrol agent is at peak in the area of plant protection with great potential to replace the chemical control measures now prevalent. The present study aims at understanding the morphology, host specificity and phage preparation in order to effectively fight potentially adapting R. solanacearum. The phages were collected from varied agro climatic zones of India were screened for high temperature tolerance at 48°C, 50°C and 52°C for 1 hr and 2D-PAGE analysis revealed accumulation of high molecular weight acidic proteins. Identification of these proteins by MALDI-TOF and their role in thermotolerance are discussed in this paper.

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Pseudomonas fluorescens SP007s reduces plant infection and increases γ-aminobutyric acid in seed infected by a complex pathogens of rice

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Field experiments using seed and foliar treatments to test efficacy of Pseudomonas fluorescens SP007s against brown spot (caused by Helminthosporium oryzae) and dirty panicle (caused by complex pathogens) were conducted. SP007s significantly reduced disease severity of both diseases, promoted plant growth and increased yield (P = 0.05). Expression of different defense-related enzymes including superoxide dismutase, guaiacol peroxidase, β-1,3-glucanase, peroxidase and phenylalanine-ammonia-lyase was detected at 1 day post SP007s spray which inversely correlated with the reduction of AUDPC. Treatment of naturally infected seeds (dirty panicle caused by H. oryzae, Fusarium semitectum, Cercospora oryzae, Curvularia lunata, and Alternaria panckwii) with SP007s enhanced pathogen reduction, seed germination, shoot, and root length with 92, 22, 36, and 25% respectively. Populations of all causal pathogens were significantly and consistently suppressed in treated seeds over 3-month investigation. Increased γ-aminobutyric acid (GABA) accumulation in only treated seeds that positively correlated with pathogen reduction was also observed. The result supports GABA mechanism involving seed resistance induction against populations of infested seeds.

The effects of salinity on Phytophthora ramorum viability and infectivity

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Phytophthora ramorum, a threat to Eastern U.S. forests, has been found in waterways outside the boundaries of infested ornamental nurseries. Very little is known about what factors are conducive to its survival and sporulation in water. Water collected from various sources with different salinity was used to better understand what effect salinity has on the life cycle of P. ramorum and infection of tissue. Water samples were collected from natural bodies of water with conductivities of 5.6, 30.5, 32.3, and 35.3 in May 2010. The water samples were added to cups containing P. ramorum-infested sand (1,000 chlamydomospores/cc). Rhododendron leaf disks were placed on the water surface for 1 week at 20°C and then plated on Phytophthora-selective medium (PARPH+V8). Very few leaf disks (<5%) were infected at the three highest conductivity levels while 100% infection occurred at 5.6 mS. Similarly, Rhododendron leaf disks were placed on the surface of different salt solutions added to P. ramorum-infested sand at two chlamydomospore levels (100 and 1,000/cc) for 1 week and plated on PARPH+V8. The leaf disks were exposed to the conductivity levels of 10.3, 26.5, 36.0, 57.2, and 67.9 mS. The disk infection rates at 100 spores/cc were 61.1, 23.1, 3.3, 0, and 0%, respectively, while infection rates at 1,000 spores/cc were 100, 70.0, 55.6, 2.2, and 0%, respectively. This research helps to better understand the survival and factors affecting infectivity of P. ramorum.

Increased strawberry production in Florida over a generation is associated with adoption of favorable arthropod management practices

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Strawberry production in Florida, U.S.A. has increased from about 1,600 ha. valued about $65 million in 1978 to about $362 million in 2010. The entire production is for fresh-market winter sales along the U.S.A. east coast. The principal arthropod pests include the two-spotted spider mite (Tetranychus urticae Koch), southern and fall armyworms (Spodoptera eridania (Cramer) and S. frugiperda (J.E. Smith)), Frankliniella bispinosa flower thrips, melon aphid (Aphis gossypii Glover), two Drosophila spp. fruit flies and three or more species of sap beetles (Nitidulidae), although several others and what effect salinity has on the life cycle of P. ramorum and past 34 years, management measures have incorporated new practices such as scouting and applying remediation as ecological conditions warrant, use of Phytoseiulus persimilis predator for control the main pest two-spotted spider mite, elimination of all chlorinated hydrocarbon insecticides, elimination of 73% of the 11 previously used organophosphate insecticides, introduction of eight new species of beneficial insects, development of integrated pest management strategy which included introduction of 18 modern insecticides largely environmentally and toxicologically more benign than the insecticides they replaced. These changes have been associated with increased production, reduced impact on the environment, and enhanced worker and consumer safety.

Disease progress of thousand cankers disease in Oregon

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Thousand cankers disease has spread throughout Oregon since first observed in the early 90’s. Symptomatic, mature black walnut (Juglans sp.) trees are harvested for valuable lumber with the assumption they will rapidly decline and die. Disease progress was documented for 60 trees from 11 locations in the Willamette Valley from Sep 07 to Jul 10. The walnut twig beetle vector (Pityophthorus juglandis) and causal pathogen (Geosmithia morbida) were confirmed in each general location. The amount of canopy with dieback symptoms was recorded for each tree in Sep 07, Sep 08, Aug 09 and Jul 10. At the Jun 10 rating 15 trees had higher canopy dieback ratings, 36 had similar ratings and 8 trees had lower dieback ratings when compared to Aug 09. Trees with dieback ratings had an average increase of 6.4%, with a range from 5 to 20%. Trees with lower ratings had an average decrease of 6.2% ranging from 5 to 10%. At the Jul 10 rating for trees on which data had been collected in Sep 07, 17 trees had higher canopy dieback ratings, 26 had similar ratings and 8 trees had lower ratings. Trees with higher ratings had an average increase of 17.2%, with a range from 5 to 70%. Trees with lower ratings had an average decrease of 6.3% ranging from 5 to 15%. Although some trees seem to die quickly, the vast majority die back very slowly, if at all. Based on these observations, disease progression in trees with thousand cankers disease is a slow process in Oregon.

Species profile and genetic variation of Fusarium isolates sampled from koa trees in Hawaii

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The native Hawaiian koa (Acacia koa) trees suffer from wilts and diebacks which were considered to be caused by Fusarium oxysporum. To determine the causal agents of the koa wilt and their genetic variation, 92 isolates of Fusarium spp. were recovered from rhizosphere soil, roots and branches of wilted or die-backed koa plants, and characterized by sequence analyses of partial translation elongation factor (EF-1a) gene, the beta-tubulin gene and the nuclear ribosomal intergenic spacer region (IGS-rDNA). Based on sequence identity, 50 of the Fusarium isolates were identified as F. oxysporum. Other species identified includes F. solani, F. pseudococcinatum, F. equisetii, F. boothii, Nectria rigidiuscula and Neonectria castaneiucola. The genetic variation of the Fusarium isolates was further evaluated using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. The four AFLP primer pairs used (E-AC/M-CG, E-AT/M-CC, E-AA/M-CT, and E-AA/M-AT) generated polymorphism among the 92 fungal isolates. However, the nine SSR primer pairs derived from F. oxysporum only produced amplicons in the 50 F. oxysporum isolates and 18 of the other Fusarium isolates. Cluster analysis using the DNA markers indicated that isolated distinct clusters corresponding to the Fusarium species identified. The results indicate that F. oxysporum was the predominant species colonizing the roots of wilted koa plants and exhibited a certain level of genetic variation.

Antibiosis by Pantoaea agglomerans biocontrol strain E325 against Erwinia amylovora on apple flower stigmas

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Phytopathology 101:S146

Pantoaea agglomerans E325, the active ingredient in a commercial product for fire blight control, was previously shown in vitro to produce a unique alkaline- and phosphate-sensitive antibiotic specific to Erwinia amylovora. Antibiosis was evaluated as a mode of antagonism on flower stigmas using two antibiosis-deficient mutants. On King’s medium B, mutants E325Ada1 and E325Ada2 have stable smooth-butyrous or hypermucoid colony morphologies, respectively. The parental strain was E325 exhibits phenotype plasticity with predominantly hypermucoid colonies accompanied by slower-growing, smooth-butryous colonies. Mutants were tested against E. amylovora on stigmas of detached flowers of crab apple (Malus Mandshurica) in growth chambers and apple (Malus domestica) in the orchard. Epiphytic fitness of the antibiotic-negative mutants was similar or greater than the parental strain as determined by relative area under the population curve (RAUPC). In laboratory and orchard trials, both mutants had significantly lower inhibitory activity against the pathogen (i.e., less reduction of E. amylovora RAUPC)
compared to the parental strain. E325 and the mutants caused similar decreases in pH in a broth medium, indicating that acidification, which was previously reported as a possible mechanism of pathogen inhibition on stigmas, is not directly related to antibiosis. In this study we provide the first evidence for E325 antibiosis involved in *E. amylovora* growth suppression on apple flower stigmas.

**Genetic characterization and distribution of mating type genes in *Sclerotinia homoeocarpa* populations**

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Phytopathology 101:S147

Dollar spot, the most economically important disease of turfgrass worldwide, is caused by the filamentous ascomycete *Sclerotinia homoeocarpa*. The objective of this research is to characterize the genetics and distribution of mating type (MAT) genes in *S. homoeocarpa* populations. In an early draft genome assembly of *S. homoeocarpa*, the MAT locus was found to contain regions with similarity to the MAT1-1-1 genes, containing an alpha motif, and the MAT1-1-5 genes in the available genomes of *S. sclerotiorum* and Botrytis cinerea. Primers anchored in the flanking DNA lypse and cytoskeleton assembly genes were used to amplify and sequence the MAT1-2 idiomorph, which contained a high mobility group-box motif with similarity to MAT1-2-1 genes. The MAT locus in *S. homoeocarpa* is similar to that of *B. cinerea* with respect to gene orientation and the presence of a truncated portion of MAT1-2-1 flanking the MAT1-1 idiomorph. However, unlike *B. cinerea*, elements of the MAT1-2-3 gene and a deleted portion of MAT1-1 were not detected in the MAT1-2 idiomorph in *S. homoeocarpa*. In a limited survey, 55 of 121 isolates of *S. homoeocarpa* from North America and 3 of 49 isolates from Asia, Europe, and South America were determined to contain the MAT1-1 idiomorph. A multiplex PCR assay is currently being developed to rapidly screen worldwide populations of the pathogen. Data developed from this study will be useful in population studies of *S. homoeocarpa*.

**Development and characterization of microsatellite markers for *Sclerotinia homoeocarpa***

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Phytopathology 101:S147

*Sclerotinia homoeocarpa* causes dollar spot, the most economically important disease of turfgrass worldwide. The objective of this research is to develop and use microsatellite markers in population studies of *S. homoeocarpa*. Microsatellites were initially isolated using a bead capture enrichment protocol, and additional repeats were identified in silico from an early draft genome assembly of *S. homoeocarpa* using the Tandem Repeat Database. Microsatellites with sufficient flanking sequence to the end of the read or to adjacent repeats were deemed suitable for primer design. Next, candidate loci were examined for polymorphisms by Sanger sequencing. Candidates containing indels in the flanking region or compound polymorphisms were discarded as not usable. From the genome data, 6,075 microsatellites were identified based on minimum thresholds of repeat number, copy number, and perfection of repeat units. Two of 31 candidate loci from the enrichment protocol were selected as usable. Of the 791 candidate loci identified in silico, 60 to date 5 usable loci have been selected and 42 have been discarded. Two to four alleles per locus have been found among a select group of cool- and warm-season isolates from four continents. Multiplex PCR protocols using fluorescently-tagged universal primers are being developed to enable rapid genotyping. These microsatellites will be useful in determining the diversity and structure among worldwide populations of *S. homoeocarpa*.

**The effect of plant activators on salinity-induced predisposition in tomato to Phytophthora root rot and bacterial speck disease**

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Phytophthora capsici or with the bacterial speck pathogen *Pseudomonas syringae pv. tomato* (*Pst*). Root treatment with the plant activators induced resistance in leaves to *Pst*, but not to *P. capsici*. Salicylic acid-deficient NahG transgenic plants displayed enhanced susceptibility and salt-induced predisposition to both pathogens. Pretreatment of roots with Tiadinil reduced salinity-induced predisposition to *Pst*, but not to *P. capsici*. Actigard and Tiadinil-induced ABA in roots and shoots to levels observed with salt stress, suggesting that plant activators can override predisposition observed in some diseases associated with stress-induced ABA.

**Temporal dispersal patterns of *Sclerotinia sclerotiorum* ascospores during canola flowering**

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Phytopathology 101:S147

The temporal patterns of *Sclerotinia sclerotiorum* ascospores dispersal in canola field were studied at two North Dakota locations between 2005 and 2007. Seven-day volumetric spore samplers were used to monitor airborne ascospore population while electronic data loggers recorded hourly information on air temperature, relative humidity, soil temperature, and soil moisture under the conala canopy. Ascospore dispersal occurred during single period that lasted 4-6 hours. In 2005 and 2007 most ascospore were collected between 10 am and 1 pm; however, in 2006, a drier than normal year, most were collected between 2am and 7am. In 2005 and 2007 the first sharp increase in ascospore dispersal was preceded by a 10-unit drop in relative humidity from close to saturation, and an increase in air temperature of 5°C. In 2006, however, no significant changes in relative humidity, which remained around 90%, or air temperature, which hovered around 15°C, were recorded prior to the start of the discharges. These nighttime discharges lasted two hours longer than daytime discharges. Daily discharges, daytime and nighttime, started when relative humidity was ≥90% and air temperature was around 10–16°C. Multiple peak-days, days with mean ≥20 ascospores/m³, were recorded in 2005 and 2007, but none were recorded in 2006. Peak-days were associated with preceding periods of seven consecutive days with mean relative humidity ≥85% in the canola canopy.

**Physiological and genetic differentiation of Curvularia lunata and resistance evaluation on corn Curvularia leaf spot in northeast of China**

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PEOPLES REP OF CHINA
Phytopathology 101:S147

Curvularia lunata, 26 isolates of mainly isolated from corn Curvularia leaf spot in 15 different geographic regions in Jilin, Liaoning and Heilongjiang province, which were characterized by different host and random amplified polymorphic DNA(RAPD). Two kinds of variation may have a certain correlation. No close correlation was found between genetic diversity and geographic orgins of isolates. The strains for test were identified as five pathogenic types by differential host which higher pathogenic types was distributed widely in Northeast, therefore that could be considered as the dominant pathogenic group. However highest pathogenic type was Baicheng in Jilin Province. The weakly pathogenic types was mainly distributed in Baishan and Lishu in Jilin Province. Eight primers were employed for RAPD analyses of 26 isolates. Out of the 77 RAPD markers, 71 polymorphic markers (P = 92.2%) were obtained. Most strains with highest and higher pathogenicity was clustered together, however strain with weak pathogenicity was clustered at lower similar level. 200 maize cultivars were collected to assess their resistance to *Curvularia lunata* by artificial inoculation at the seedling and adult stages in the field. No immune genotypes were found. Highly resistant were 3%, resistant were 4% and intermediate resistant were 33%, and immediately susceptible were 23.5 and susceptible were 36.5%.

**Genetic analysis of gene conferring resistance to wheat stripe rust in Lanka05**

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Phytopathology 101:S147

Stripe rust, caused by *Puccinia striiformis* f. sp. triticii, is one of the most serious diseases of wheat in the worldwide. China is the largest epidemic region in the world and severe losses in grain yield have been reported. The disease-resistant variety is controls the wheat stripe rust to be most economical, the securety and effective method. To identify new resistance genes is significant in wheat breeding. Lanka05 is a famous variety that has resistance to stripe rust, for identifying the genetically characterize, F1, F2, F3 and BC1F1
progenies from the cross of Lankao 5/Chinese Spring were tested with PST races CYR29, CYR30, CYR31, CYR32, SU11-4 and SU11-11 in the greenhouse, respectively. Genetic analysis showed that resistance of Lankao5 to SU11-4, CYR31 and CYR32 were conferred by two dominant genes, to SU11-11 conferred by one dominant gene, to CYR30 conferred by one dominant gene and one recessive gene and to CYR29 conferred by two recessive genes. Lankao 5 as resistant resource and its genetic information should be useful in breeding resistant cultivar to stripe rust.

** Races of *Exserohilum turcicum* and evaluation of maize cultivars on the resistance to Northern corn leaf blight in Jilin Province of China

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Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Pass.) Leonard & Suggs, is an important foliar disease of corn in Northeast of China. The disease was observed and sporadic occurrence at first in Northeast of China in 1899. Gene-for-gene relationships have been demonstrated for E. turcicum on maize. 15 physiological races including 0, 1, 2, 3, 12, 13, 1N, 2N, 3N, 12N, 13N, 123N, 123N, 2N were have been identified according to virulence formula (Leonard,1989) in Northeast of China. It is quite complex race component in Jilin province of Northeast of China, and there were not dominant races as well as in mid-erosionous production areas of maize. Especially, new virulent races can overcame all multi gene of Ht resistance. 123N (0/H1, H2, H3, H1N) were prevalent in the middle and west of Jilin province. 100 lesions lamina were collected from 7 maize-growing areas of Jilin province, and identifying race 123N with the frequency of 11%. 269 maize cultivars were collected from seeds market in Northeast of China, and evaluated for resistance to northern corn leaf blight under the level of greenhouse. The result showed that the susceptible varieties with the frequency of 64.31%, mid-susceptible varieties occupy 21.56%, mid-resistance varieties occupy 10.40% and resistance varieties only 3.72%.

**Nematidal activity of two components from the broth filtrate of *Aspergillus niger* Y-61**

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PEOPLES REP OF CHINA
Phytopathology 101:S148

Application biological pesticides are more concern in China. A fungus strain of *Aspergillus niger* Y-61 was isolated from farming soil of Beijing and against *Meloidogyne incognita*. Using the method of immersion, The hatching rate of J2 induced by space mutation was highly susceptible. H4 to isolate GD0193 was controlled by a single isolates. They were demonstrated to show high level of field resistance tested in system. The mutated lines H4, H11 and D69 conferring improved resistance to PST. They were identified as race 123N with the frequency of 11%. 269 maize cultivars were collected from seeds market in Northeast of China, and evaluated for resistance to northern corn leaf blight under the level of greenhouse. The result showed that the susceptible varieties with the frequency of 64.31%, mid-susceptible varieties occupy 21.56%, mid-resistance varieties occupy 10.40% and resistance varieties only 3.72%.

**Novel heat-stable protein elicitor from *Alternaria tenuissima* activates plants resistance and growth**

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Phytopathology 101:S148

This paper describes the discovery of a new microbial protein elicitor named PeaT1 that significantly enhances plant disease resistance and crop growth. PeaT1 obviously suppresses TMV on tobacco leaf, grey mould on tomato and greatly increases plant growth in rice, tomato, tobacco and cucumber. We have identified the gene sequence of PeaT1 and described the processes of purification and identification through electrophoresis, anion exchange chromatography and mass spectrometry. Action research results suggest that PeaT1 activate chlorophyll nutrition and formation uptake by up-regulating a series of related genes using the rice oligo microarray. The molecular mechanism of PeaT1 inducing disease resistance in tobacco was likely through the systemic acquired resistance pathway mediated by salicylic acid and the NPR1 gene.

**Comparative analyses of endogenous small RNAs in *Sclerotinia sclerotiorum* and *S. trifoliorum* by 454 Titanium RNA sequencing**

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Endogenous small RNAs (sRNAs) of *Sclerotinia sclerotiorum* and *S. trifoliorum* were compared to gain insight into the biology of the two closely related plant pathogens. Random samples of 53 unique sRNAs of *S. sclerotiorum* and 55 unique sRNAs of *S. trifoliorum* had, respectively, 221 and 229 target loci in the *S. sclerotiorum* genome database. More than half of the sRNAs targeted at exons in both species. The sRNA target loci were not evenly distributed among the 37 superfecogonits of the *S. sclerotiorum* genome. The sRNA target loci from *S. trifoliorum* had the highest frequency per superfecogon nucleus in Superfecoil 35, whereas Superfecoil 56 had the highest frequency of sRNA target loci of *S. sclerotiorum*, suggesting that superfecoils 35 and 36 are hot-spots for sRNA biogenesis. Four sRNAs were found in both species (four pairs). Two pairs targeted five orthologs of the same Tj2 retrotransposon. The other two targeterred exons, one of which is a microRNA-like sRNA. However, BLAST searches found no similar sequences in the microRNA database (miRNA-BASE), suggesting unique gene with different mechanisms of sRNA biogenesis than other euakaryotes. Two putative dicer-like (DCL) genes were identified, and DCL-2 was expressed at higher levels than DCL-1 in both species. Similarly, among three putative Argonaut protein gene transcripts AGO1 is the highest expressed Argonaut protein gene in both species, suggesting prominent roles of DCL-2 and AGO1 in sRNA biogenesis of *Sclerotinia* species.

**Comparative transcriptome analysis in *Sclerotinia sclerotiorum* and *S. trifoliorum* by 454 Titanium RNA sequencing**

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Phytopathology 101:S148

*Sclerotinia sclerotiorum* and *S. trifoliorum* are two closely related devastating plant pathogens. Extensive research has been conducted on *S. sclerotiorum* and its genome sequences are available. To take advantages of the genomic information of *S. sclerotiorum*, we compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. Total transcripts of both species during vegetative growth were extracted and sequenced using the latest 454 Titanium RNA sequencing technology. A total of 23325 unique transcripts with average length of 534 nt (12.5 mb genome coverage) were obtained from *S. sclerotiorum*, whereas 21214 unique transcripts with average length of 509 nt (10.8 mb genome coverage) were obtained from *S. trifoliorum*. About 80% of the unique transcripts of both species were found in the *S. sclerotiorum* genome database. More than half of the transcripts were found between the DNA regions that are not considered as coding regions, and 15 of those transcripts were the same in both species, suggesting they are functional. Twenty-eight contigs (transcripts with more than one read) of *S. trifoliorum* were not found in the *S. sclerotiorum* genome database. Additionally, differences in expressed genes involved in pathogenesis like oxalate biosynthesis and endopolygalacturonases were detected between the two species.

**Rice mutated lines showing improved resistance to *Magnaporthe oryzae* induced by space mutation**

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Phytopathology 101:S148

The space mutated offspring from Zhonger Ruanzhan were evaluated on resistance to rice blast and analyzed on their resistant inheritance and R genes in system. The mutated lines H4, H11 and D69 conferring improved resistance to rice blast were 0 tested by isolate GD0193 in greenhouse. They were not found in field resistance tested in a natural nursery for 5 successive cropping seasons, while the wild-type was highly susceptible. H4 to isolate GD0193 was controlled by a single dominant gene and two independent dominant genes controlling its resistance to isolate GD0874. The R gene to isolate GD0193 in H4 has been finely located in the long arm of rice chromosome 11, linked to markers RM224 and
RM27360 with \( \approx 1.04 \) cM and 1.2 cM respectively. One of the R gene to GD0874 in H4 was located in a region on the short arm of chromosome 1, where no other blast R genes has been reported. This could be attributed to mutagenesis of the seeds, which gave rise to new resistance.

Investigating the genetic structure of *Phytophthora capsici* populations

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*Phytophthora capsici* is a destructive soilborne pathogen that infects economically important vegetable crops. The objective of this study was to investigate the genetic structure of 255 *P. capsici* isolates assigned to predefined host, geographical, mfenoxam sensitivity and mating type categories. Isolates from six continents, 21 countries, 19 U.S. states, and 26 host species were genotyped for four mitochondrial and six nuclear loci. Bayesian clustering revealed some population structure by geographic origin and mfenoxam sensitivity with some clusters occurring more or less frequently in particular categories. Bayesian clustering, split networks, and statistical parsimony genealogies also detected the presence of non-*P. capsici* individuals in our sample corresponding to *P. tropicalis* and isolates of a distinct cluster closely related to *P. capsici* and *P. tropicalis*. Our findings of genetic structuring in *P. capsici* populations highlight the importance of including isolates from all detected clusters that represent the genetic variation in *P. capsici* for development of diagnostic tools, fungicides, and host resistance. The population structure detected will also impact the design and interpretation of association studies in *P. capsici*. This study provides an initial map of global population structure of *P. capsici* but continued genotyping of isolates will be necessary to expand our knowledge of genetic variation in this important plant pathogen.

The genetic structure of *Pseudoperonospora cubensis* global populations

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*Pseudoperonospora cubensis* is a destructive foliar pathogen that infects economically important Cucurbitaceous crops in the United States and worldwide. In this study, we investigated the genetic structure of 465 *P. cubensis* isolates from three continents, 13 countries, 19 U.S. states, five host species and spanning 28 years. Isolates were assigned to predefined host, geographic and year categories and genotyped for two mitochondrial and five nuclear loci. Bayesian clustering resolved six genetic clusters and suggested some population structure by geographic origin and host, as some clusters occurred more or less frequently in particular categories. Since genetic structure has been detected in *P. cubensis* populations, it is important to include isolates that represent the genetic variation in *P. cubensis* when developing diagnostic tools, fungicides, and resistant host varieties. The population structure detected should also be taken into account when designing and interpreting association studies in this pathogen. While this study provides an initial map of global population structure of *P. cubensis* when developing populations studies, future genotyping of additional isolates would be useful to determine population structure within specific geographic regions or across a wider range of hosts.

The phenomics of rice blast: Using extensive nutritional profiling to understand how the devastating plant pathogen *Magnaporthe oryzae* causes disease

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The filamentous fungus, *Magnaporthe oryzae*, responsible for rice blast disease, destroys 10–30% of the world’s rice crop annually. Although its pathobiology has been studied for many years, hardly anything is known about nutrient acquisition from the host and the underlying physiology of the fungal *M. oryzae* strain. We initially suited for intro study because we could not culture away from its host plant, it is amenable to rapid gene functional analysis, and both *M. oryzae* and rice have sequenced genomes. Nitrogen and carbon utilization play an important role in many aspects of fungal biology especially in pathogenesis. The aim of this study is to identify key regulators of nutrient acquisition that influence pathogenesis. To facilitate our understanding of these processes, we have extensively tested our mutant strain collection on different carbon and nitrogen sources. Our results are presented in heat map form, which uses color to represent colony diameter and growth for accurate comparisons of nutrient utilization among and between different mutant strains. The data demonstrate that non-pathogenic mutant strains have a widely different nutritional profile compared to the wild type. This supports the hypothesis that some nitrogen and carbon utilizing capabilities can be important indicators of pathogenesis in the rice blast pathogen. This study is the first step in a high-throughput gene functional analysis using nutritional profiling for pathogenic gene discovery.

Raspberry latent virus a plant reovirus that is aphid transmitted in a replicative persistent manner

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*Raspberry latent virus* (RPLV), is a newly characterized reovirus found in commercial raspberry fields in the Pacific Northwest (PNW). Phylogenetic analyses showed that RPLV is related most closely to *Rice ragged stunt virus* (RRSV), the type member of the genus *Oryzavirus*. RRSV and all members of plant reoviridae are transmitted by species of leaffoppers in a replicative persistent manner. After several failed attempts to transmit RPLV using leaffoppers, *Amphorophora agathonica*, the common raspberry aphid in the PNW, was tested as a vector of RPLV. RPLV was detected in aphids after a 12h-acquisition period using quantitative RT-PCR. Using a standard curve generated for quantifying RPLV, it was shown that the virus titer in aphids continued to increase after the acquisition period even when aphids were maintained on healthy plants with successive transfers onto fresh healthy plants every two days. This suggests that the virus replicates in the vector. Serial transfer of aphids to healthy plants demonstrated that the virus has a 7-day latent period in the aphid before it can be transmitted. A low percentage of plants tested positive for RPLV, 60 days post-inoculation, using aphids that tested positive for the virus, suggesting that aphids are inefficient vectors of this virus. Further experiments showed that RPLV is not transmitted transovarially to the next generation. To our knowledge this is the first report of an aphid transmitted plant reovirus.

Significant increase in titer of *Raspberry bushy dwarf virus* when present with *Raspberry leaf mottle virus* and its effect on raspberry plants

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Phytopathology 101:S149

*Raspberry bushy dwarf virus* (RBDV) has been attributed as the causal agent of the disease. Recently, the identification of two new viruses: *Raspberry leaf mottle virus* (RLMV) and *Raspberry latent virus* (RPLV) in northern Washington (WA) and British Columbia, where crumbly fruit is more prevalent, suggested the existence of a new virus complex responsible for the increased severity of the disease. In efforts to determine the role of the new viruses on the crumbly fruit, ‘Meeker’ plants containing single and mix infections of RBDV, RLMV, or RPLV were used and developed to establish field trials. Plant growth during the first year was significantly reduced in plants infected with all three viruses and the combination RBDV/RLMV when compared to control and singly-infected plants. Quantitative RT-PCR tests revealed that the titer of RBDV was increased 800-fold when mixed with RLMV compared to RBDV in single infections. In addition, a survey of RPLV and RLMV in WA and Oregon revealed that RLMV is present at very high incidence (up to 100% in 5-year old fields) in northern WA; whereas the incidence in southern WA and Oregon, where crumbly fruit is not a problem, was considerably lower (40% in 8-year old plantings). These findings open the possibility that crumbly fruit disease could be managed by targeting RLMV’s vector, the aphid *Amphorophora agathonica*.

Soybean susceptible leaves response to *Fusarium virguliforme* toxin in a manner resembling an incompatible interaction

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Sudden death syndrome is an important disease, caused by *Fusarium virguliforme*. This fungus colonizes soybean roots causing rot, and releases a phytotoxin that is translocated to leaves causing interveinal chlorosis. In this study, we report on an Affymetrix analysis measuring transcript abundances in resistant (PI657.374) and susceptible (Essex) leaves from the same plants used to study gene expression in roots (Radwan et al., 2011, PMPI) upon infection by *F. virguliforme*. Analysis of the leaf response to *F. virguliforme* infection versus mock inoculated plant identified 2671 transcripts as being differentially expressed. Gene expression analysis has led to a working
hypothesis that the fungal toxin activates HR-defense pathways of susceptible leaves in a manner that resembles an incompatible interaction. The response was slow and led to disease induction instead of defense. Molecular markers related to senescence and cell death were induced in susceptible leaves reflecting in part the role of HR in disease symptom development. On the other hand, soybean resistant leaves may employ a lipid biosynthesis pathway to reduce the damage fallout from fungal toxin. Cross comparison of gene expression between leaves and roots indicated that while root employ mechanisms to restrict the fungal infection, leaves employ different mechanisms to reduce the toxicity of the fungal toxin. Changes in small RNA levels between inoculated and mock treated samples were also studied and will be presented.

A 14-3-3 protein appears to be required for establishing normal nodule formation in soybean

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Phytopathology 101:S150

The soybean genome contains 18 members of 14-3-3 proteins that play key functional roles in many critical physiological pathways that are regulated by phosphorylation. Transcriptomic and proteomic analyses of soybean inoculated by Bradyrhizobium japonicum revealed that a 14-3-3 transcript and protein was induced in inoculated roots versus control. To investigate the role of the 14-3-3 during the establishment of the symbiotic relationship between the root and B. japonicum, we used RNAi to silence the 14-3-3 transcript using Agrobacterium rhizogenes-mediated root transformation. The transformed roots exhibited reduced numbers of mature nodules. Inoculated 14-3-3 silenced roots contained large numbers of arrested nodule primordia and empty nodules instead of mature nodules. In addition, electron microscopy images showed that in the empty nodules the host cytoplasm was absent; in addition all membranes, but the symbiosome membrane, were gone. There are two highly similar paralogs of the 14-3-3 gene in soybean that was targeted for silencing. Specific differentiating primer pairs were designed to establish the expression patterns of each of these paralogs. Although these two paralogs most likely descended from a common ancestral DNA sequence, q-PCR suggested that the Glyma05g29080 transcript was induced preferentially. The soybean genome contains 18 members of 14-3-3 proteins that play key functional roles in many critical physiological pathways that are regulated by phosphorylation. Transcriptomic and proteomic analyses of soybean inoculated by Bradyrhizobium japonicum revealed that a 14-3-3 transcript and protein was induced in inoculated roots versus control. To investigate the role of the 14-3-3 during the establishment of the symbiotic relationship between the root and B. japonicum, we used RNAi to silence the 14-3-3 transcript using Agrobacterium rhizogenes-mediated root transformation. The transformed roots exhibited reduced numbers of mature nodules. Inoculated 14-3-3 silenced roots contained large numbers of arrested nodule primordia and empty nodules instead of mature nodules. In addition, electron microscopy images showed that in the empty nodules the host cytoplasm was absent; in addition all membranes, but the symbiosome membrane, were gone. There are two highly similar paralogs of the 14-3-3 gene in soybean that was targeted for silencing. Specific differentiating primer pairs were designed to establish the expression patterns of each of these paralogs. Although these two paralogs most likely descended from a common ancestral DNA sequence, q-PCR suggested that the Glyma05g29080 transcript was induced preferentially.

RT-PCR detection and partial characterization of Prunus necrotic ringspot virus isolates occurring in Iran

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The main areas for stone fruits production in Iran were surveyed for the occurrence of Prunus necrotic ringspot virus (PNRSV) during the April to October of 2008 to 2010. Leaf samples from 544 Prunus spp., Peach (P. persica), apricot (P. armeniaca) and plum (P. domestica) trees showing symptoms of virus infection were collected from commercial stone fruits and tested for the occurrence of PNRSV using DAS-ELISA and RT-PCR. Our study revealed a higher incidence and diversity of virus in the Tehran (19.6%) compared with the Fars (14.9%) and Golestan (11.4%) provinces. The infection levels for single species in each province were: Fars (peach, 10.6%; apricot, 17.1% and plum, 16.6%); Tehran (peach, 26.08%; apricot, 18.6% and plum, 10.9%) and Golestan (peach, 15%; apricot, 2% and plum, 7.5%) respectively. The peach isolate of PNRSV was differentiated from the apricot and plum isolates by nine differential host species. Electron microscopy examination showed spherical virions with ca 29-32 nm in diameter. All isolates had molecular weight of coat protein subunits of 29 kDa, determined by western blotting method. Three primers (VP990.91 and VP96) were used to amplify movement protein (MP) gene of three Iranian isolates of PNRSV isolated from Peach, apricot, and plum trees. Amplicons of the correct size (~280 bp) for the genes obtained from all the examined isolates of PNRSV. Most of the PNRSV isolates were identified as members of group PV32, none of the isolates belonged to group PV96 and PE5.

Analysis of Citrus Huanglongbing-associated Candidatus Liberibacter strains from Pakistan

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Citrus Huanglongbing is a serious disease known to cause significant damage to citrus industries worldwide. HLB has been known to be present in Asia for at least a century and one of the earliest records of the disease dates to early 1900s on the Punjab region of Pakistan. Molecular analysis of the isolates of HLB associated Candidatus Liberibacter asiaticus (LAS) is of importance for understanding the disease. Isolates of LAS from several regions of Punjab region from the main commercial cultivar, Kinnow mandarin, were selected. Seven different genomic regions of LAS were analyzed from the selected isolates. We included regions from different locations of the bacterial genome to better understand the extent of variability that may exist in the Candidatus Liberibacter strains from Pakistan. Preliminary results indicated the presence of at least two populations of LAS in the symptomatic plant samples. In the variable regions, the sequence of Pakistan isolates showed a significant number of differences when compared to the completely sequenced psy62 LAS strain from Florida.

Small RNAs of Magnaporthe oryzae, and the role of different sRNA biosynthetic genes on pathogenicity

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The Rice Blast fungus, Magnaporthe oryzae, causes serious disease to rice and other cereal crops worldwide. The deep sequencing analyses of small RNAs (sRNAs) from various M. oryzae mycelia exposed to different physiological stress conditions revealed the presence of more than 37 million total genome matched reads mapping to intergenic regions, coding sequences, retrotransposons, inverted repeats, tandem repeats and other repeats of the genome with more than half of the reads are from intergenic regions. The 24-nt class of sRNAs was predominant, likely reflecting a high degree of heterochromatic siRNAs. Based on the matching genomic region, sRNAs are divided into several classes, and characteristics of these classes are analyzed in detail. We also made targeted deletions of sRNA biosynthetic genes resulting in mutants having phenotypes different from wild type. The phenotypic analyses of several mutants are discussed.

Characterizing whitefly species and/or biotypes vectoring geminiviruses on peppers in Indonesia

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Whitely transmitted Geminivirus is one of the key diseases on peppers in Java, Indonesia. Pesticides are applied as frequently as 40 times over the course of the crop without significant benefit. One of the key steps in developing integrated management strategies for the vectors is to accurately identify the vector species and/or biotypes transmitting geminiviruses. Whitely samples were collected from pepper, other crop and weed plants in pepper production systems of Java. The collected whitely samples were identified as Bemisia tabaci, Trialeurodes vaporariorum and Aleyrodic dyspersion based on the morphological characters. Since B. tabaci has several biotypes, the biotypes were confirmed using the partial mitochondrial COI gene sequences. The COI specific primer pairs (C1-J-2195 and L2-N-3014) amplified a PCR product of approximately 900 bp size. The sequence alignment and editing resulted in a consensus sequence of 750–800 bp across all samples. The phylogenetic analysis showed two biotypes of B. tabaci: Asia I and Asia II. More than 90% of the samples clearly grouped with the biotype Asia I. This was already documented in few other studies in Indonesia and our study also confirmed the results. However, two samples from Ipomoea aquatica and Trialeurodes sp. grouped with Asia II biotype. Thus, it could be hypothesized that the predominant biotype in hot pepper production systems of Java is Asia I, and the development of management strategies should specifically focus on this biotype.
Incidence of criniviruses in multiple crops in Costa Rica

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Members of the whitfly-transmitted genus Crinivirus within the family Closteroviridae are emerging threats to both vegetable and fruit production worldwide. Recent surveys for criniviruses using symptomatology, RT-PCR, and real-time quantitative RT-PCR were performed in vegetable crops in the Cartago province of Costa Rica, one of the most important agricultural areas in the country. We identified Beet pseudo yellows virus in field-grown cucurbits and Tomato chlorosis virus (ToCV) in field- and greenhouse-grown tomatoes, and greenhouse-grown sweet peppers using virus-specific primers. In addition, newly discovered natural hosts of ToCV, including multiple species of common weeds growing adjacent to tomato nurseries and in production greenhouses in Cartago, were identified and may serve as virus reservoirs for agricultural crops. The most prevalent whitfly species found were the greenhouse whitfly (Trialeurodes vaporariorum) and biotype B of Bemisia tabaci. Using molecular tools we identified, for the first time in the agronomical region of Alfaro Ruiz, Costa Rica, the presence of the insecticide-resistant exotic Q biotype of Bemisia tabaci in greenhouses where tomatoes and peppers are grown. The results of our studies provide a better understanding of the epidemiology of criniviruses and their insect vectors in Costa Rica and will be used to develop improved disease management strategies.

Multiplication and movement of Xylella fastidiosa in Australian native plant species

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Xylella fastidiosa Wells is a xylem-limited plant pathogenic bacterium that causes diseases in numerous host species including food and fodder crops, ornamentals and weeds. The pathogen is vectored by insects, predominantly Homalodisca vitripennis Germar (Hemiptera: Cicadellidae). Xylella fastidiosa, not yet detected in Australia, is native to the Americas and is considered to be highly invasive. Australian climatic conditions are favourable for pathogen establishment and there is a need to develop the capacity for rapid detection and containment of an incursion, including knowledge of host plant species to target monitoring. In Riverside, California, twelve Australian native plant species were inoculated with X. fastidiosa and assayed for the pathogen after ten months using culturing and PCR to determine host status, symptom development, systemic spread and persistence of the pathogen over winter. The host status of several Australian native plant species will be presented and these host species may act as reservoirs from which further spread of the pathogen can take place should it reach Australia. The implications of these findings will be discussed and placed in an Australian invasion context.

Detection of ‘Candidatus Liberibacter asiaticus’ in psyllid and citrus hosts in Pakistan and analysis of psyllid populations

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The occurrence of Diaphorina citri and a description of huanglongbing (HLB) or greening disease were reported by Husain and Nath from the Punjab area of Pakistan in 1927. The disease has been “lived with” for several decades. Recent research effort focus on improvement of the breeding programs system and genetic study of the psyllid vector and the bacterium associated with HLB, ‘Candidatus Liberibacter asiaticus’ (LAS). Psyllid populations were monitored by use of yellow sticky traps from twelve orchards in Sargodha region. Population peaks of psyllids were observed in April-May and Oct-Nov, with the highest populations occurring in Oct-Nov. Upon testing the psyllids for presence of LAS using qPCR, the bacterium was detected in psyllids from all 11 locations indicating that HLB is widespread in the region. However, psyllid samples collected during the months of June/July tested negative by qPCR for the bacterium. Presence of the bacterium associated with HLB in plants was demonstrated by testing the DNA extracted from plant samples using qPCR and conventional PCR, and further confirmed by sequencing. Plant samples collected in June/July showed very low titers of LAS.

Postharvest control of gray mold of blackberry caused by Botrytis cinerea with preharvest applications of fungicides in Michoacan México

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Phytopathology 101:S151

Grey mold of blackberry caused by Botrytis cinerea is one of the most important diseases in Michoacan México. After harvest infected fruits are covered with gray mycelium and spores that make the fruit unsuitable for marketing. The objective of this research was to evaluate the preharvest effect of chemical, biological and biorational fungicides against gray mold of blackberry in Michoacán México. The experiment was conducted in the growing seasons of 2009 and 2010 in a commercial plot located at Zaracuaro, Michoacan. Commercial formulations of Copper sulfate, captan Bacillus subtilis, Hydrogen Dioxide, Grapefruit Seed extract, Harpin protein, Fenhexamid, Ipodione, Cypuridin+Fludioxonil and Boscalid+Pyraclostrobin were sprayed in a full season program, during bloom or preharvest. In order to determine disease incidence harvested fruits were incubated at room temperature for 7 days. In both years of testing there were significant differences among treatments (P < 0.001). Two sprayings of Fenhexamid, Ipodione, Boscalid+Pyraclostrobin and Cypuridin+Fludioxonil either during bloom or before harvest (green to red berry) complemented with 2 sprayings of Captan gave the lower disease incidence in postharvest. Full season programs based on Copper Sulfate, Captan, harpin protein and Grapefruit seed extracts provided good to moderate control of gray mold, but Hydrogen Dioxide did not provide acceptable disease control in the 2 years of testing.

Responses of maize (Zea mays L.) near isogenic lines carrying Wsm1, Wsm2 and Wsm3 to three viruses in the Potyviridae

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Phytopathology 101:S151

Genes on chromosomes six (Wsm1), three (Wsm2) and ten (Wsm3) in the maize inbred line Pa405 control resistance to Wheat streak mosaic virus (WSMV), Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV) (3). Near isogenic lines (NIL) carrying one or two of these genes were developed by introgressing regions of the respective chromosomes into the susceptible line Oh28, and tested for their responses to WSMV, MDMV and SCMV in the field and greenhouse. F1 progeny from NIL x Oh28 were also tested. Wsm1, or closely linked genes, provided resistance to all three viruses, as determined by symptom incidence and severity. Wsm2 and Wsm3 provided moderate resistance to both WSMV, MDMV and SCMV, but significantly increased resistance in plants with one Wsm1 allele. NIL carrying Wsm1, Wsm2 and Wsm3 had similar SCMV resistance in the field, but NIL with Wsm2 and Wsm3 were not resistant in the greenhouse. Addition of Wsm2 to Wsm1 increased SCMV resistance in the field. For all viruses, symptom incidence was higher in the greenhouse than in the field, and relative disease severity was higher in the greenhouse for WSMV and MDMV. An MDMV (Wi isolate and the Ohio SCMV infected the Wsm7 NIL, while the Ohio MDMV and Seehausen SCMV isolates did not. Our results indicate that the three genes, or closely linked loci, provide virus resistance. Resistance is influenced by interactions among the genes, the virus species, the virus isolate and the environment.

Are plant communities shaped by fungal root endophytes?

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Plant communities are the result of a complex interplay among plants, symbiosis and the environment. Dark septate endophytes (DSE) of the Phialocephala fortinii s.l. – Acephala applanata species complex (PAC) are ubiquitous fungal root colonizers of a wide variety of woody plant species but their ecological role is largely unknown. Sterile seedlings of Betula pendula (birch) and Picea abies (spruce) in monoculture and in mixed culture were exposed to four PAC strains, either singularly or paired in all possible
combinations at 18°C and 23°C. Plant and fungal biomass was determined after four months. Colonization by PAC reduced biomass gain of either host. One of the strains was more virulent than any other strain to spruce but not to the birch. Biomass gain of spruce was slightly reduced and that of birch enhanced at higher temperature. Virulence of pathogenic strains was reduced in some strain mixtures, highlighting the importance of high genotype diversity on small spatial scale. The effect of PAC on plant biomass gain depended mainly on the mixture of PAC genotypes and the host plant species. Fungal biomass was higher in spruce than in birch and at lower temperature. Our results indicate that the presence of particular PAC genotypes can have an impact on the result of competition among plant species and thereby contribute to plant community formation.

Identification of solanaceous and non-solanaceous species as hosts of Stemphylium solani isolates in Brazil

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Stemphylium solani is one of the causal agents of the gray leaf spot of tomatoes in Brazil and it has been reported on distinct hosts in the Solanaceae and in other botanical families. In this work, we tested the Koch’s postulates for the new S. solani isolates and evaluate a collection of solanaceous and non-solanaceous accessions for their reaction to S. solani isolates. Identification as S. solani was based upon morphology and the ITS sequence. All the isolates were virulent when inoculated on their original hosts and on tomato cultivar ‘Ponderosa’. In addition the pathogen was reisolated from the symptomatic plants, fulfilling the Koch’s postulates. In the host range assay, 72 accessions (13 families, 30 genera, and 58 species) were inoculated with four (tomato, gilo, eggplant, and Capsicum) isolates. The following species were confirmed as hosts: potato, peppers (Capsicum spp.), gilo (Solanum aethiopicum var. gilo), and eggplant. This updated host list contains new reports for Brazil: C. chineise, C. frutescens, Physalis sp., Nicandra physaloides, S. paniculatum, S. palinacanthum, S. betacea, and Datura stramonium. Non-solanaceous hosts were confirmed: cotton, Ocimum basilicum (Lamiaceae), Zinnia elegans (Compositae), Tabebuia impetiginosa and S. serratifolia (Bignoniaceae). Differential interaction of host accessions and fungal isolates were observed for eggplant, N. physaloides, and S. paniculatum, suggesting the presence of physiological races in S. solani.

Silicon and its interaction with fungicide on the control of anthracnose in susceptible and resistant sorghum lines

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This study aimed to evaluate the effect of silicon (Si), and its interaction with fungicide, to decrease the severity of sorghum anthracnose in a field experiment carried out in a silicon-deficient soil area. The experimental design was a split-plot design, with four lines, 30 genotypes, and 58 species of plants. The whole plot corresponded to calcium silicate (AgroSilício) and lime (-Si) treatments with fungicide spray. The application of calcium silicate decreased AUAPC by 42 and 35%, respectively, for non-spray and spray of fungicide. Fungicide application decreased AUAPC by 44 and 37%, respectively, for non-spray and spray of fungicide. The application of calcium silicate contributed to decrease AUAPC by 44 and 37%, respectively, for non-spray and spray of fungicide. Fungicide spray decreased AUAPC by 42 and 50%, respectively, for lines BR-009 and BR-008. For non-spray and spray of fungicide, AUAPC was reduced, respectively, by 88 and 90% for line BR-008 and 87 and 88% for line BR-009. The application of calcium silicate contributed to decrease AUAPC by 44 and 35%, respectively, for lines BR-009 and BR-008. The application of calcium silicate decreased AUAPC by 44 and 37%, respectively, for non-spray and spray of fungicide. Fungicide spray decreased AUAPC by 39 and 50%, respectively, for non-spray and spray of fungicide. The application of calcium silicate contributed to decrease AUAPC by 44 and 37%, respectively, for non-spray and spray of fungicide. Fungicide spray decreased AUAPC by 39 and 50%, respectively, for non-spray and spray of fungicide.

Cassava’s immunity suppression mediated by Type III effectors of Xanthomonas axonopodis pv. manihotis

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Xanthomonas axonopodis pv. manihotis (Xam) relies on the Type III Secretion System to translocate effector proteins, involved in the suppression of plant defense, to cause Cassava Bacterial Blight. Plant defenses can be induced by recognition of Microbial Associated Molecular Patterns, triggering a response known as PTI or basal defense. This line of defense involves responses such as strengthening of cell wall through callose deposition. Nonetheless this basal defense can be overcome by effectors of pathogens. A second defense level is related to Recognition of Microbial Associated Molecular Patterns, triggering a response known as ETI or inducible defense. This line of defense involves responses such as strengthening of cell wall through callose deposition and gene expression associated with the development of systemic acquired resistance. Our results support the majority of Xam’s effectors in our test study can suppress plant immunity. As this is an artificial system, it will be interesting to demonstrate the impact of the suppression of these effectors in cassava plants.

Detection and localization of Undifilum oxytropis fungi in locoweed tissues

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Locoweed hosts an endophyte Undifilum oxytropis that produces the alkaloid swainsonine, which is responsible for locoism in grazing animals. The livestock industry in western United States is strongly affected by locoism caused by ingestion of locoweed tissues. Untreated locoweed tissues are toxic to livestock and endophyte-infected locoweed can be identified by fluorescence microscopy. Our results suggest that the majority of Xam’s effectors in our test study can suppress plant immunity. As this is an artificial system, it will be interesting to demonstrate the impact of the suppression of these effectors in cassava plants.

Systemic resistance phenomena from an evolutionary perspective

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Systemic acquired or induced resistance has been studied for several decades. Aside from theoretical interest the phenomena may have practical application in disease management. The practical use of SAR/SIR for disease management raises the issue of the potential fitness cost of resistance priming. We contend that the on-going debate over this issue is a red herring which arises from two sources. First, physiological studies which focus on fitness, but not on fitness of the complete sense, may present a misleading impression of the relative importance of measured differences between induced and non-induced plants; fitness may be constrained by covariance between components. We highlight well known results from population ecology which define the appropriate experimental framework for measuring the impact of resistance priming on host fitness. Secondly, we contend that the physiological and biochemical resistance mechanisms linked with systemic resistance phenomena are a distraction from the truly significant aspect, which is the ability of plants to detect environmental cues linked to an increased

RpfG, a two-component regulator with a CheY-like receiver domain attached to a HD-GYP, a protein of which little is known but seems to have an important role inside the signal network. The aim of this study was to manually annotate the rpf cluster in Xam genome and to assess the interaction of rpfG with two proteins, XC 0420 and XC 0249 corresponding homologues in Xam using the yeast two-hybrid system. According to a Bayesian analysis performed, Xac did not form a monophyletic group with the other X. axonopodis pathovars, in contrast with the pathovars of the species Xanthomonas campestris, Xanthomonas oryzae and Xanthomonas vasicola that were grouped together. Additionally, we found a direct interaction of proteins XC 0420 and XC 0249 with RpfG, showing that these proteins are relevant for the functioning of the system network in Xam. These results are critical in the elucidation of the function of the quorum sensing system in Xam, which has not been studied in detail yet.
probability of disease and to respond to those cues. We describe results from
information theory and theoretical ecology which support this view. Empirical
studies using *Pinus/Arabidopsis* and *Arafidopsis* *Alternaria* as experimental
long- and short-term systems, respectively, are being combined with modeling
to investigate our hypotheses.

Remote sensing for detection of Rhizoctonia crown and root rot in sugar
beet fields

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Rhizoctonia crown and root rot (RCRR) of sugar beet is caused by
*Rhizoctonia solani* AG-2-2. Disease ratings are based on subjective, visual
estimates of root rot severity (0–7 scale). Remote sensing was evaluated as an
alternative method to assess RCRR. Field plots of sugar beet were inoculated
at the 10-leaf stage with *R. solani* AG 2-2 IIIB at a range of inoculum
densities in 2008 and 2009. Data were collected for 1) hyperspectral
reflectance from the sugar beet canopy and 2) visual ratings of RCRR in 2008
at 2, 4, 6, and 8 weeks after inoculation (WAI) and in 2009 at 2, 3, 5 and 9
WAI. 100 randomly selected plants and five wideband vegetation indices (VIs) were
assessed; the wideband optimized soil adjusted vegetation index (OSAVI)
provided the best overall fit with disease severity ratings. Values of VIs
were constant until 25–50% of the root surface rotted and then decreased
significantly as disease severity increased. RCRR also was detected using
airborne, color-infrared imagery at 0.25 m. Remote sensing detected RCRR,
but not before initial appearance of visual symptoms. In 2010, OSAVI image
analysis of a series of aerial images obtained with a multispectral camera were
used to identify areas within commercial fields that were symptomatic of
RCRR. Fields were then ground-truthed for RCRR, potential insect
populations and soil nutrient problems. Analysis of aerial-obtained
multispectral imagery has promise in identifying areas of RCRR in the field.

Defining the interactome underlying Sudden Death Syndrome of soybean

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Phytopathology 101:S153

The soil-borne ascomycete Fusarium virguliforme is the causal agent of
Sudden Death Syndrome (SDS) of soybean, a devastating disease that has
recently emerged as one of the most important diseases of soybean in the U.S.
F. virguliforme colonizes the roots of soybean causing severe root rot, and
also produces a potent phytotoxin that is translocated to leaf tissue leading to
foliar necrosis. Despite the widespread importance of this disease, surprisingly
little is known about the molecular mechanisms underlying pathogenesis. The
goal of this project was to identify plant and fungal genes involved in
pathogenesis based on expression profiles. To this end, total RNA from
infect ed and healthy roots was extracted, and digital gene expression data were acquired
with next-generation sequencing techniques. Highly expressed fungal genes were
predicted to regulate signal transduction, secondary metabolism, and
carbohydrate hydrolysis. Selected candidate genes involved in pathogenesis
and host defense were further evaluated with quantitative PCR. This study
provides a molecular perspective on SDS and identifies candidate fungal
genes for future functional studies.

Where does it come from?: Determining initial inoculum for dollar spot

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Phytopathology 101:S153

Dollar spot, caused by Sclerotinia homoeocarpa (F.T. Bennett), is one of the
most devastating diseases of turfgrasses worldwide. Understanding the basic
biology of this pathosystem is critical because management has been
complicated by the loss of broad spectrum fungicides and advent of fungicide
resistance. In this study, we determined the source of initial inoculum, seedborne or as mycelium within host tissue. Plant material
obtained by taking 40 soil cores adjacent to and three inches from dollar spot
infection centers on creeping bentgrass, was collected in the late fall and early
spring. Roots and shoots were separately removed from each core and plated
on semi-selective media. Colonies resembling *S. homoeocarpa* were
subcultured and identified using morphological and molecular techniques.
Results indicate that *S. homoeocarpa* survives predominantly on shoots at the
margins of infection centers. Seed assays consisted of four treatments: Non-
stere lized (NS)/non-infected (NI), Sterilized/infected (I), Bleached/NI, and
NI/NI. Seeds from each treatment were plated on semi-selective media
individually and as a slurry. Colonies resembling *S. homoeocarpa* were
identified as above. The highest rates of isolation were obtained from
Bleached/NI and NS/NI seed, suggesting that *S. homoeocarpa* can colonize seed and likely infiltrates beyond the surface. This research indicates two possible
sources of initial inoculum for dollar spot that may be targeted as a means of
delaying disease onset.

Deployment of rapid diagnostic tools for Phytophthora on horticultural
crops in Central America

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Phytopathology 101:S153

Plant disease is a limiting factor in agricultural production in Latin America
due to high rainfall conditions and the presence of a diversity of plant
pathogenic microorganisms. Losses estimated to be as high as $30 billion per
year. There designed to provide early prediction capabilities for important plant disease threats in the region. We are
working with collaborators including, FIH in Honduras, Universidad de
Costa Rica, CATIE, The World Cacao Foundation, DOLE Foods, and the
Organization of Tropical Studies to conduct surveys of Phytophthora species
on horticultural crops in the region.

A Lucid key to the common Phytophthora species

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Phytopathology 101:S153

The Key to the Common Phytophthora species (Lucid v 3.4) is a matrix-based
computerized identification key and includes important morphological and
molecular characters that are useful for identification of 55 common
Phytophthora species. A set of 20 features are used to make a correct species
identification. The user enters responses to known character state options into
a Lucid v3.4 key and the correct species is identified. Illustrations of each feature
state are included in the key. The main features included in the key are:
axial features, sexual structures, and chlamydomspore, hyphae and cell wall
characteristics. The user can read an illustrated “Fact Sheet” on each species. A
cross-linked glossary of terminology is included in the “Fact Sheet”. In
addition, a DNA Search function of ITS and Barcode of Life (5′ end of the coxl gene) sequences for each species can be queried. The key was created to provide
practitioners, regulatory personnel and teachers with easily accessible tools to distinguish common species based on a number of important morphological
and molecular characteristics and is now available from

Development of a PCR-RFLP method to rapidly identify common
entomopathogenic fungi infecting soybean aphid

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Phytopathology 101:S153

Soybean producers face pest pressures from soybean aphid (*Aphis glycines*
Matsumura) and foliar fungal diseases (e.g. brown spot, frogeye leaf spot, 
*Cercospora* leaf blight). Soybean aphid is an extremely economically
important pest in many parts of the United States and Canada, causing
yield reduction of 14–50% if left untreated. Foliar disease impacts on yield are
not well established, but fungicides are frequently applied prophylactically.
Conidiothrubos thomboideus and *Pandora neaphidis* are two common fungal

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pathogens of soybean aphid in North America. In vitro testing of the impact of several fungicides on these aphid pathogens has shown reduced and, in some cases, complete inhibition of germination and infectivity. Aphid-pathogenic fungi are a source of natural soybean aphid population control and are potentially valuable biocontrol agents. A cultivation independent PCR-based diagnostic tool has been developed for detection of *P. neosphacid* in the environment, but there is no similar tool for monitoring *C. thottoboides*. Universal primers were used to amplify the ITS rDNA regions of both species and ITS-RFLP analysis was used to identify fingerprints to distinguish between the two species. ITS-RFLP analysis was successfully performed on fungal isolates and artificially infected soybean aphids. The analysis was then used to determine the incidence of *P. neosphacid* and *C. thottoboides* in naturally occurring populations of soybean aphid during the 2009 and 2010 growing seasons.

Open access online database of powdery mildews (Order Erysiphales) in Puerto Rico

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Phytopathology 101:S154

An open access online database was developed containing information of powdery mildews (Ascomycetes: Order Erysiphales) of Puerto Rico. In the tropics, there is very limited knowledge of these species that affect our crops. From 2010 to 2011 we conducted a series of surveys to examine powdery mildew diversity in the island. Tissue samples were collected from more than 15 plant hosts and have been analyzed revealing the presence of at least 6 different powdery mildew genera. The database is focused to offer a friendly guidance to the diagnostic of powdery mildew pathogens occurring in the island. It includes morphology using light and scanning electron microscopy and phylogenetic analysis of DNA-sequence profiles and alignments- in order to standardize genetic identification of Erysiphales occurring in the Caribbean. The specific identification of these pathogens will help enforced quarantine regulations to stop the introduction of new species of pathogens in an already fragile island ecosystem.

A novel vitivirus isolated from *Ribes* species in Alaska

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A new virus of domesticated black and red currants (*Ribes nigrum L. and R. rubrum L.*) was isolated in 2008 from a home garden on the Kenai Peninsula near Homer, Alaska. Leaf symptoms consisted of mosaic with vein-clearing and chlorotic spots. *Nicotiana benthamiana*, *C. quinoa* and *C. amaranthicolor* Coste, and Reyn. developed mosaic or local lesions (latter two) when inoculated with either leaf sap or partially purified particle preparations. Protein and ds-RNA extracts from inoculated *N. benthamiana* that exhibited mosaic symptoms contained a tentative coat protein ~22 kDa and a prominent dsRNA ~7.5 kb, respectively. The dsRNA was sequenced using conventional and Illumina platform sequencing. A complete genomic sequence of 7,729 nt determined that the virus belonged to the genus *Vitivirus* and was most similar to *Grapevine virus E*. The *Ribes* vitivirus had amino acid sequence identities of 49% with the RNA dependent RNA polymerase, 44% with the movement protein and 30% with the putative nucleic acid binding protein. This ORF has been found in all the *Ribes* virus isolates that exhibited *trans* symptoms but transmitted curtoviruses, one of which, *Beet milder curtovirus*, was previously found in *Ni. benthamiana*. The *Ribes* vitivirus is currently the only one known to carry a long intergenic region that is absent in all GVE isolates reported thus far.

Identification of curly top virus infection in Jalapeño pepper in Chihuahua, Mexico

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Curly top is a serious problem in many irrigated crops in the dry semi-arid climates in North America. The disease is caused by a complex of leafhopper-transmitted curtoviruses, one of which, *Beet mild curly top virus*, was previously found in *N. benthamiana*. Mexico is the last few years, sporadic symptoms similar to curly top disease were observed in Jalapeño pepper in the South Central area of Chihuahua State. The symptoms featured stunted, yellowing plants scattered in the otherwise healthy looking pepper stand. Affected leaves were brittle, showed upward curling, and distinct green vein pattern with interveinal yellowing. In June and August of 2010, two field surveys were conducted in Jalapeño pepper stands. A total of 94 pepper plants were subjected to TAS-ELISA using the recently developed polyclonal curly top virus status of the collected samples was determined by RT-PCR. Of the collected 94 samples from pepper plants showing stunting and yellowing 11 were found *fected* by *PVYNTN* recombinants. Based on the combination of biological, serological, and molecular characteristics, this recombiant strain from Mexico may belong to the PVY2 strain group represented by the isolate PVY-L26.
Streptomyces was applied in rhizosphere, Ganoderma in phyllosphere and pathogens in selected leaves by syringe infiltration. Ganoderma showed total inhibition in vitro of all isolates after 24 hours and 89.8% of them after 48 hours. Streptomyces inhibited all isolates within a range of 6.59–100% after 24 hours. Plants inoculated with the pathogen in greenhouse showed the disease symptoms but when they were treated with the antagonists, a significant infection reduction was observed, being Ganoderma the most effective. Streptomyces reduced pathogen population but infection was not diminished in the same proportion. Treatments with the antagonists combined with the pathogens showed the highest plant height, and chlorophyll and biomass content. This is the first study that deals with biocontrol of the bacterial spot of pepper in Chihuahua, showing its potential effectiveness under field conditions.

Spatial characterization of favorable climate conditions for soybean rust progress on current and future scenarios in Brazil

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Current maps of spatial distribution of areas with favorable climate conditions for soybean rust progress have been developed for Brazil using data from the average monthly temperature and relative humidity obtained from the Climate Research Unit. Data refer to historical averages of these variables from 1961 to 1990. The A2 and B2 future scenarios were designed for the decades of 2020, 2050, and 2080 from six climate models of the Intergovernmental Panel on Climate Change. Based on them, maps of future scenarios for favorability of rust occurrence on different regions in Brazil were developed. The maps of future scenarios were prepared according to estimates of severity and rate of rust progress based on multiple regression models. Regarding the current condition, it was observed that the rust progress was favorable mainly due to better temperature and rainfall conditions in the main growing soybean regions. By contrast, during the dry season (Jul. and Aug.), rust progress was not favorable due to low temperatures. Analyzing the future projections, there will be an increase in areas highly favorable for rust in the next decade. In the years 2050 and 2080, there will be a decrease in favorability in some growing regions with a decline for A2 scenario with the highest temperature increase. Regarding to the climate, there will be reduction in areas favorable for soybean rust progress as compared to the current period. Financial support: CNPq.

The burden of truth: Visual representations of genetic engineering and genetically modified organisms in the online media

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Groups for or against genetic engineering have used visuals to capture public attention, stir emotions, and mobilize support—tasks that are now easier to accomplish through the web. Although images of biotechnology saturate the media, do they accurately portray the science and the process? What is their tone toward GE and GMOs? A content analysis of web images—photographs, illustrations, charts, videos, and other graphic representations—of genetically modified organisms in the online media, a seven-day period was conducted. The results show an abundance of visuals in personal and special interest group sites, stock photo and cartoon banks. Images with a negative valence trounced those with a positive tone in frequency and intensity. The visuals presented a range of perspectives on GE, but many failed the accuracy test. Recurring inaccuracies fall along the following lines: incorrect representations of the process; almost non-existent depiction of biosafety protocols; overly artistic images that obscure the rationale for GE; the stigmatization of Monsanto; monsters result from mixing plant and animal genes; (6) scare tactics exaggerate risks to human health; and limited comparative analyses. The findings indicate that people’s ability to produce attractive graphics has outstripped knowledge of how to use them well. The result is a glut of captivating visuals clashing the information stream and impairing audiences’ understanding of an important innovation.

Evaluation of Arabidopsis thaliana as a model host for Xylella fastidiosa

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Phytopathology 101:S155

Pierce’s disease of grapes and almond leaf scorch are agronomic diseases caused by the bacterium Xylella fastidiosa. To date, progress determining mechanisms of host plant susceptibility, tolerance or resistance has been slow, due in large part to the long generation time and limited available genetic resources for grape, almond and other known hosts of X. fastidiosa. To overcome many of these limitations, Arabidopsis thaliana has been evaluated as a host for X. fastidiosa. A pin-prick inoculation method has been developed to infect Arabidopsis with X. fastidiosa. Following infection, X. fastidiosa multiplies robustly and can be detected by microscopy, PCR and isolation. The ecotypes Van-0, LL-0 and Tsu-1 all allow more growth of X. fastidiosa strain Temecula than the reference ecotype Col-0. Various X. fastidiosa strains also show differential growth in Arabidopsis. Affymetrix ATH1 microarray analysis of inoculated vs. non-inoculated Tsu-1 reveals gene expression changes that differ greatly from changes seen after infection with apoplastic colonizing bacteria. Many genes responsive to oxidative stress are differentially regulated while classic pathogenesis-related (PR) genes are not induced by X. fastidiosa infection.

Validation of a single nucleotide polymorphism genotyping method for Wheat streak mosaic virus

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Phytopathology 101:S155

Wheat, one of the most economically valuable crops in the United States, ranks first in crop exports. Wheat pathogens threaten both national and international commerce, and could be deployed intentionally to impact trade streams. Attribution of such agricultural crimes will require new forensic tools that are more stringent and targeted. An assay using primer elongation with fluorescent dideoxynucleotides at potential single nucleotide polymorphic (SNP) sites was developed and tested for its ability to discriminate reliably among plant pathogen strains using Wheat streak mosaic virus (WSMV) as a model. Fifteen SNPs were identified in the coat protein (CP) and helper-component protease (HCPro) regions of the genome and a unique primer was designed for each. Consistent, distinguishable SNP fingerprints, consisting of patterns of chromatographic peaks, were obtained using six strains and eighteen field isolates of WSMV. The sensitivity of the assay was determined using a synthetic plasmid of the WSMV CP and HCPro genes. The specificity to WSMV was demonstrated by testing an exclusivity panel consisting of near-neighbors to WSMV. The SNP genotyping method appears to be an appropriate method for forensic discrimination among WSMV strains.

Effects of hot water treatment for seed disinfection and seed germination in rice

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Phytopathology 101:S155

Bakanae disease caused by the Fusarium fujikuroi is a significant disease in rice growing region of the world. This disease is spread by seeds infection so seed disinfection need for prevent disease spread primarily. But environment friendly farming system doesn’t permit using any chemicals for seed disinfection. Thus cold-hot water treatment method has been widely using as a seed disinfection for controlling seed infectious diseases in environment friendly farming. Hot water treatment method was developed by observing 5 min of cold water treatment and it’s control effects for bakanae disease was equal to compared with cold-hot water treatment. Appropriate proportion of seeds and water in hot water treatment method was 250 g in 2,000 ml for stable seed disinfection. In germination test with 60 rice cultivars by hot water treatment, most of rice cultivars were showed safe germination in 60°C for 20 minutes. In eight night cultivars Goonjung1, Samgwangbyeo, Saongwangbyeo, Sinunbong1, Ungwangbyeo, were showed over than 20% reduction of germination ratio in 60°C for 15 minutes and in 60°C for 20 minutes. Seed germination ratio was decreased 40 to 50% when pre-soaked rice seeds in plane water over 3 hours before hot water treatment in seed disinfection but represented normal seed germination when pre-soaked in the brine.

First report of Alternaria malii on apples in Brazil

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Phytopathology 101:S155

A species of Alternaria was isolated from leaf spots on Gala apples (Malus domestica Borkh.) collected from four orchards in Paraná State, southern Brazil, in 2007 and in 2010. The leaf spots were circular, tan to brown, 2 to 5 mm in diameter and were often initially bordered by a purple halo. The fungus was identified as A. malii Roberts based on conidial morphology and dimensions in vitro. A pin-prick inoculation method has been developed

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Gala following inoculations in the laboratory with a conidial suspension at 104 conidium mL⁻¹ and the fungus was resolicited from the lesions. This is the first report of A. mali from Brazil. The importance of this disease is not known because it is often found on leaves severely affected with Glomerella leaf spot, caused by Colletotrichum gloeosporioides, C. acutatum and G. cingulata.

Species identification of the causal agent of Eutypa dieback of grapevine in northeastern U.S. and southeastern Canadian vineyards


Phytopathology 101:S156

Eutypa dieback of Vitis grape is caused by the Ascomycete fungus Eutypa lata. The pathogen infects grapevine through wounds, and cause wood canker and dieback symptoms. E. lata has been identified in all major grape production areas in the world. The first report of Eutypa dieback from the northeastern United States identified the causal agent as E. lata. However, our recent studies questioned the species identity of the causal agent of Eutypa dieback in these regions. Our objectives were to: 1) survey Eutypa-affected vineyards in northeastern U.S. (CT, MA, MI, NY, OH, RI) and Ontario, Canada and measure disease incidence; 2) identify the Eutypa species using multi-gene phylogeny, microsatellite analysis and secondary metabolite profile; 3) determine the pathogenicity of the Eutypa species recovered. Our results indicated that the incidence of Eutypa dieback increased with the density of vineyards. Based on phylogenetic analyses of three nuclear loci, we identified E. lata, E. laevata, and two new undescribed species that are closely related to E. lata. E. lata was only found in two vineyards (Rhode Island and Ontario) suggesting that these collections may represent introductions from outside the eastern U.S. and Canada. All Eutypa species produced phytotoxins and were pathogenic on Vitis labruscana ‘Concord’ and V. vinifera ‘Chardonnay’.

A new approach to manage phytoplasma diseases: Field treatments with resistance inducers to control grapevine Bois noir

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Phytopathology 101:S156

Bois noir is one of the main phytoplasma diseases of grapevine in the Mediterranean basin. It induces severe loss of production due to the early drying of most of the bunches of grapes on the plant, and to stunted plant growth and general decline. At present, there are no known methods of containing this disease. The aims of this study are to investigate the spread of Bois noir in vineyards, to record the physiological changes of the infected plants, and to promote symptom remission or recovery through spraying of the experimental material. We investigated the potential to delay the beginning of July drying of symptomatic plants by about 50%. This increase in plant resistance provides an innovative approach to the management of grapevine Bois noir, and contribute to the opening of new possibilities for the containment of phytoplasma disease.

Characterizing microbially communities of potato common scab suppressive soil using pyrosequencing

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Phytopathology 101:S156

Potato common scab (PCS), caused by Streptomyces spp. is an annual production concern for commercial potato growers. A naturally occurring PCS suppressive soil has been identified in Michigan. To characterize the microbial community structure of the disease suppressive soil, pyrosequencing was employed. Soil was sampled from disease conducive and suppressive fields in the same location, and total genomic DNA was extracted from these soils. The pyrosequencing approach was used to analyze amplicon libraries from polymerase chain reaction amplification of a phylogenetically informative region (variable across taxa) within the 16S RNA gene. Data was analyzed using operational taxonomic unit (OTU)-based, taxon-based and phylogenetic-based methods. The number of OTUs (10% dissimilarity) identified from disease conducive soil, disease suppressive soil and shared between sites was 565, 859, and 300 respectively. 26.69% of OTUs were shared between conducive and suppressive soil and the total number of OTUs was 1,124. Additionally phylogenetic analysis of pyrosequencing tag data of samples from conducive and suppressive soil samples found that the total number of phyla, classes, orders, families and genera set was 20, 49, 87, 173 and 335 respectively. The results of this study will provide information for potato crop production on how to enhance beneficial soil microbial communities and will have significant effects on plant health and in soil disease suppressiveness, particularly in the case of PCS.

AS1-261: A potential non-fumigant alternative to methyl bromide


Phytopathology 101:S156

A novel compound, AS1-261, is in development as a pre-plant soil treatment for broad-spectrum pest control. The material had activity against fungi, oomycetes, and nematodes when tested in vitro. In greenhouse assays, no phytotoxicity was observed on tomato or bell pepper when transplanted 5 days after soil treatment. Two small-scale field trials on bell pepper and tomato were conducted and a strawberry trial is underway. Bell pepper yields in small plots were similar to the methyl bromide control. Weed biomass emerging through plant holes in plots receiving the highest AS1-261 rate were slightly greater than the methyl bromide treatment, but were lower than the untreated control (UTC). In the strawberry trial, mortality of introduced inoculum of Macrophomina phaseolina was significantly increased with the high rate of AS1-261 compared to the UTC. Trichoderma colony forming units were higher in both AS1-261 treatments than in the untreated and 1,3-dichloropropene treated plots. Sting nematode numbers, while relatively low, were significantly reduced immediately after treatment and were equivalent to numbers extracted from plots treated with 1,3-D. Advantages of using this experimental material include the ability to make applications via drip irrigation with no volatile organic compounds generated, which should result in minimal worker exposure and few regulatory constraints and the broad-spectrum of activity against soilborne pests.

Genome sequence of an unassigned Citrus tristeza virus genotypic isolate from Puerto Rico reveals a trifoliate resistance breaking genotype

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Phytopathology 101:S156

The use of our recently developed genotype specific multiplex (GSM) reverse transcription polymerase chain reaction (RT-PCR) analysis left the Citrus tristeza virus (CTV) isolate B301 as an unassigned CTV genotype. The GSM RT-PCR method can detect CTV in any infected plants but failed to distinguish two other RB genotypes, CTV-B165 and CTV-B301. The RT-PCR method can detect CTV in any infected plants but failed to distinguish two other RB genotypes, CTV-B165 and CTV-B301. Sequence analysis of 26-nt terminal half region revealed that B301 shared 97% nucleotide sequence identity with RB isolate B301, which had been isolated from citrus in Puerto Rico in 1992. Biologically, B301 induces symptoms similar to mild CTV-T30 like isolates and does not induce seedling yellows or stem pitting symptoms. We hypothesized the RB isolate was a potential new RB genotype. A new isolate from Puerto Rico reveals a trifoliate resistance breaking genotype.

Characterization of the Pi-b rice blast resistance gene in the National Small Grains Collection (NSGC)

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Phytopathology 101:S156

The Pi-b gene in rice confers resistance to a wide range of races of the rice blast fungus, Magnaporthe oryzae, including race Eik that overcomes Pi-ta. In the present study, Pi-b was identified in 164 rice germplasm accessions from the National Small Grains Collection using DNA markers and pathogenicity assays. The existence of Pi-b in rice germplasm was detected by
using two simple sequence repeat (SSR) markers, RM 208 and RM 166, and a
dominant marker Pibdom derived from Pi-b. Pathogenicity assays using an
avirulent race (IE1K) and a virulent race (IB54) were performed to verify
resistance specificity of Pi-b. Among the 164 germplasm accessions
evaluated, 130 were found to contain the Pi-b gene using both SSR markers
and pathogenicity assays, although with different haplotypes. The remaining
34 germplasm accessions were found to be different in their responses to the
blast races IB54 and IE1K, suggesting the presence of Pi-b independent R
gene(s). These characterized germplasm accessions can be used for genetic
study and marker-assisted breeding for improving blast resistance in rice.

IPM programs for winter wheat in Oklahoma: A team approach to
manage insects, diseases and weeds

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Phytopathology 101:S157

Wheat is a multi-million dollar crop in Oklahoma, grown on more than 2
million hectares. Insects, plant diseases and weeds all provide major
challenges for producers to profitably grow winter wheat. Scientists and
Extension professionals and resources from Oklahoma State University, the
USDA’s Agricultural Research Service, and the National Institute of Food and
Agriculture join in partnership with Oklahoma’s wheat producers to
successfully address many of these pest problems in a collaborative “team
approach”. This presentation describes how the teams are organized and
outlines current successes and future challenges that are being addressed.
These challenges include development of winter wheat varieties that are
resistant to insects and diseases, development of weed management programs
that integrate rotational cropping systems with herbicide resistant varieties,
development of insect management programs that incorporate assessment of
natural enemy activity in conjunction with pest activity and methods for
effectively delivering information to Oklahoma’s producers.

Previous reports of bacterial diseases on crucifers attributed to
Pseudomonas syringae pv. maculicola were caused by P. cannabina pv.
alisalensis

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Phytopathology 101:S157

Pseudomonas cannabina pv. alisalensis (Pca) causes bacterial blight on
crucifers, which can reduce crucifer yields and has resulted in economic losses
in the U.S. Prior to the late 1990s Pca was not distinguished from the pepper
spot pathogen of crucifers, Pseudomonas syringae pv. maculicola (Psm),
although these organisms have since been found to have distinct host ranges
and to belong to different species. The objective of this research was to
determine whether recent and historical reports of crucifer diseases attributed
to Psm were in fact caused by Pca. Bacteria identified as Psm from disease
outbreaks worldwide were compared to Psm and Pca. DNA fragment banding
patterns generated by repetitive-PCR using the BOXA1R primer distinguished
Pca from Psm and demonstrated that some of the pathogens previously
identified as Psm were Pca. Additionally, the putative Pca strains and the
pathotype P. cannabina sensitive to bacofen PHS1 while Psm was not.
The identity of the putative Pca strains was confirmed through host range
evaluations. The putative Pca strains and the Pca pathotype were pathogenic
on radish (cv Comet), rapini (cv Sorrento) and oats (cv Montezuma) but Psm
was not. Correctly identifying and distinguishing these pathogens is crucial
for developing effective management strategies and preventing pathogen
spread. The outlined suite of assays represent methods effective in
distinguishing these previously commingled pathogens.

Impact of soybean cyst nematode on Rhizoctonia root and crown rot of
sugar beet

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Phytopathology 101:S157

Soybean cyst nematode (SCN; Heterodera glycines) penetrates sugar beet
roots and likely creates small lesions. This damage might affect seedling
disease caused by Rhizoctonia solani, an important pathogen of sugar beet in
the Red River Valley. The objectives of this research were to determine if
SCN penetrates sugar beet roots under field conditions and if SCN increases
root disease caused by R. solani. Field soil free of SCN was infested with
SCN eggs at three egg density levels and sugar beets were grown under field
conditions and the roots harvested at 4, 8 and 16 weeks post planting. DNA
was extracted and PCR analysis was performed to identify SCN in the roots.

To determine if SCN increases disease 2 week old plants in growth chambers
were either inoculated with R. solani or co-inoculated with R. solani and SCN
eggs then rated for necrosis after 10 days. Results showed that SCN could be
detected in sugar beet roots grown under field conditions throughout all
time points using PCR analysis. Although there was no significant difference
in necrosis between treatments with R. solani alone and R. solani plus SCN
when data was averaged over four experiments, there were consistently higher
ratings for necrosis in each experiment when SCN was added in with R.
solani. This consistent increase in root necrosis observed in the presence
of SCN warrants continued investigation to determine the overall impact SCN
has on sugar beet seedling diseases.

Assessing the validity of diagnostic quantitative PCR assays for
Phakopsora pachyrhizi and P. meliboea

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There are 123 confirmed species in the genus Phakopsora worldwide, with 19
species reported in the continental United States. In 2002, a quantitative PCR
(qPCR) diagnostic assay was developed by Frederick et al. and currently is
being used for detecting Phakopsora pachyrhizi in spore trapping studies.
Based upon these assays, spores of P. pachyrhizi have been reported in Ohio
and other states where soybean rust has never been found. These reports may
be based upon false positives. False positives are problematic because they
can lead to unnecessary fungicide applications when there is no risk of
disease development. In 2009 a new qPCR diagnostic assay was developed
by Barnes et al. to eliminate false positive results. Both qPCR assays were tested
against other rust pathogens; however, neither of these assays was tested
against closely related Phakopsora spp. (other than P. meliboea) that are
known to occur in the continental United States. The species that we will test
include P. arthuri, P. crotonis, P. meliboea, P. nishidiana, P. pachyrhizi,
P. tecta, and an unknown Phakopsora species. We will assess the two
diagnostic assays against these Phakopsora spp.

Detection and identification of various Clavibacter michiganensis strains
using a novel isothermal nucleic acid amplification

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Clavibacter michiganensis is the causative agent for diseases in several
economically important crops. Clavibacter michiganensis subsp. sepedonicus
(Cms) causes ring rot in potatoes while Clavibacter michiganensis subsp.
michiganensis (Cmm) causes bacterial canker in tomatoes. We report here on
a novel isothermal nucleic acid amplification method for the detection and
identification of Cmm and Cms, using bacteria in culture and may lead to unnecessary fungicide applications when there is no risk of
disease development. In 2009 a new qPCR diagnostic assay was developed
by Barnes et al. to eliminate false positive results. Both qPCR assays were tested
against other rust pathogens; however, neither of these assays was tested
against closely related Phakopsora spp. (other than P. meliboea) that are
known to occur in the continental United States. The species that we will test
include P. arthuri, P. crotonis, P. meliboea, P. nishidiana, P. pachyrhizi,
P. tecta, and an unknown Phakopsora species. We will assess the two
diagnostic assays against these Phakopsora spp.

First report of Phyllactinia guttata on almonds in Lebanon

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Almond, Prunus dulcis (Mill) Webb = Amygdalus communis L., has been
cultivated for centuries in Lebanon, of a typical Mediterranean climate. In
a recent survey of diseases occurring on almond species in Lebanon, the
powdery mildew disease caused by Podosphaera pannosa was mainly
observed. However a new record of a late season powdery mildew caused by
Phyllactinia guttata (Wallr.) Lév, was detected on cultivated and wild almond
species. In the coastal area, it was observed on the cultivated almond species
P. dulcis and at the higher elevations was on the wild almonds P. korschinskii
and P. orientalis. The signs of Ph. guttata showed as white mycelium on the
lower side of leaves. The cleistothecia, 150-250 um in diameter, could be
clearly seen with the naked eye. Each cleistothecium has 8 to 12 equatorial
bristle-like appendages. Each appendage has a bulbous base 25-50 um wide
and its length is 1 to 2.0 times the diameter of the cleistothecium asccopar.
Each ascocarp contains up to 20 asci and each ascus contains 2 ascospores
ellipsoidal to ovoid in shape. The hyphae of its anamorph were thin and
persistent, conidia clavate to rhomboid belonging to genus Ovulariopsis.
These findings were consistent with those reported by Braun (1987) for the
same species. This powdery mildew species has not been reported previously
on Almonds in other countries of the Mediterranean region.

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Host specificity in *Erwinia tracheiphila* (Smith): Evidence from rep-PCR and pathogenicity assays

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Bacterial wilt of cucurbits, caused by *E. tracheiphila*, impacts all cucurbits except watermelon. Although this disease causes severe yield losses throughout the eastern U.S., little is known about the biology of the pathogen. Genomic DNA from *E. tracheiphila* strains, isolated from symptomatic cucurbit crop plants obtained from seven states, was amplified with BOXA1R and ERIC1-2 primers. Banding patterns were observed after agarose gel electrophoresis and compared among hosts. Banding patterns from strains isolated from *Cucumis* spp. plants were distinct from those isolated from the genus *Cucurbita* regardless of geographic origin. Twelve *E. tracheiphila* strains isolated from *C. melo* (melo L.), *C. sativus*, or squash (*C. pepo*) were inoculated onto leaves of 2-week-old *C. mело* L. and *C. pepo*. Wilt symptoms were assessed over two weeks, strains were re-isolated, and rep-PCR banding patterns were compared to the inoculated strain. All strains were pathogenic to both hosts. *C. mело* plants expressed wilt symptoms 4 to 5 days sooner when inoculated with *Cucumis* spp. strains than when the same strains were inoculated onto *C. pepo*. *C. pepo* plants inoculated with *Cucurbita* spp. strains expressed symptoms 4 to 5 days sooner than when the same strains were inoculated onto *C. mело* L. Banding patterns from the re-isolated strains were consistent with the originally inoculated strains. Our results suggest that *E. tracheiphila* strains are genetically diverse and that this diversity may be specific to host genus.

Identification and characterization of a new ampelovirus infecting cultivated and wild blackberries

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A novel ampelovirus from blackberry was identified recently in Mississippi and characterized in the framework of NIFA-funded Specialty Crop Research Initiative (SCRI) projects on viruses affecting blackberries in the Southeastern United States. The virus sequence was obtained from high throughput sequencing (Illumina platform) using dsRNA as template and RT-PCR to fill in gaps. The genome organization of this virus resembles that of Grapevine leafroll-associated virus 3, the type member of the genus Ampelovirus (family Closteroviridae). Amino acid sequence identities between genomic products of the blackberry ampelovirus and GLRaV-3 varied from 35% (diverged coat protein) to 65% (RNA-dependent RNA polymerase), suggesting that this blackberry virus is a new member of the genus. Phylogenetic trees, independent of method used or genomic products compared, always placed this blackberry virus closest to GLRaV-3. Preliminary survey carried out on a limited number of samples, indicated the presence of this virus in several cultivated and wild blackberry hybrids. Identification of this putative blackberry virus and evaluation of its incidence/importance in blackberry in the major blackberry-producing areas of the U.S. are the present focus of this research.

Molecular characterization of an endornavirus from *Cucumis* spp.

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Endornaviruses infect hosts in the kingdoms Plantae, Fungi and Chromista. They are efficiently transmitted vertically and generally do not induce visible symptoms. In this investigation high molecular weight dsRNA, representing the genome of an endornavirus, was isolated from an unknown melon (*Cucumis melo*) cultivar and used as a template for molecular characterization. The complete genome of the virus, provisionally named Cucumis endornavirus 1 (CueEV-1) consisted of ca 15 kbp, terminating with a stretch of cytidine residues and encoding a large precursor polypeptide containing domains characteristic of RNA-dependent RNA polymerase and glyco-syltransferase. CueEV-1 is phylogenetically closely related to endornaviruses reported from cultivated and wild Orzya spp. Preliminary studies indicate the presence of variants of this virus, or closely related endornavirus species, in several other wild and cultivated cucurbit species.

Emaravirus and cryopivirus infection of Viburnum lantanoides in the Great Smoky Mountains National Park

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Phytopathology 101:S158

Mosaic/line pattern symptoms resembling viral infections were observed on *Viburnum lantanoides* Michx. (Witch-Hobble, Bobblebush or Moosewood) in the Great Smoky Mountains National Park. Double stranded RNA extraction revealed the presence of multiple faint bands that were used as a template for further molecular investigation. Shotgun cloning of reverse-transcribed dsRNAs revealed the presence of genomic sequences of two phytopyviruses in the original sample. Genomic segments of a putative cryopivirus showed similarities with corresponding regions of Beet cryptic viruses 2 and 3 and several other cryopiviruses. The second virus identified in this study is related, distinct, from European mountain ash ringspot-associated virus and Fig mosaic virus (ca 40-42% identical amino acid contents of putative nucleocapsid and glycoprotein precursor), recognized species in the genus *Emaravirus*. Considering that *Emaraviruses* are an emerging group of viruses associated with diseases in cultivated and wild flora, the involvement of this virus in the observed symptomatology is very plausible. Further epidemiological and etiological studies are underway.

A putative novel carlavivirus associated with the disease in *Magnolia tripetala* L.

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Phytopathology 101:S158

Mosaic symptoms were observed on a specimen of an Umbrella-tree (syn. *Umbrella magnolia*) present on the campus of the Highlands Biological Station, North Carolina, in summer 2010. Transmission electron microscopy observations of partially purified preparations revealed the presence of slightly flexu-ous filamentous virus-like particles c 650-700 nm in length that prompted further study. RT-PCR performed on purified dsRNAs using general flexivirus primers indicated the presence of a virus closely related to several species in the genus *Carlavirus*, fam. Betalaviridae. The polyadenylated genome of this virus, a putative new species in this taxon tentatively denominated Magnolia mosaic virus (MagMV), is 8.6 kb long and contains six ORFs. MagMV shares ca 67% overall nucleotide sequences with the type isolate of *BiSeV*. Common amino acid content of genomic products between the two viruses vary from 65% (NABP) to 80% (TGBp-3). Virus-specific RT-PCR was developed and used to investigate the presence of this virus in different magnolia genotypes.

Practical resistance to fenhexamid *Botrytis cinerea* isolates from grapevines in New York

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Phytopathology 101:S158

Fenhexamid is a fungicide used to control *Botrytis cinerea* on grapes worldwide. Resistance appears to be of a quantitative rather than qualitative nature, with minimum EC50 values that define a resistant phenotype proposed as exceeding 0.1 mg/L by some workers and 0.4 mg/L by others. However, little is known about the degree to which isolates of these sensitivities are controlled by the material when subjected to typical application rates in the field environments. In this study, a total of 388 *B. cinerea* isolates were collected from New York State vineyards and their sensitivity to fenhexamid was examined. Morphological, physiological and genetic characteristics of 12 strains with EC50 values greater than 0.1 mg/L were defined. Four isolates whose EC50 value of fenhexamid is 0.033, 0.105, 0.318 and 1.626 were used for *in vitro* inoculation tests to gauge whether they show practical resistance. Inoculation tests using grape berries showed that pre-inoculation spray with the field rate of 1.2 mg/L controlled *B. cinerea* more than post-inoculation spray with the same rate, regardless of EC50 value of four isolates tested.

Studies on *Maize streak* virus infection and yield attributes in F1 maize hybrids

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Cultivation of tolerant genotypes remains an effective strategy against streak disease caused by *Maize streak virus* (MSV; *genus Mastrevirus*) in sub-Saharan Africa. Yield attributes in 15 MSV tolerant F1 hybrids, MSV resistance gene donor Tzi3 and MSV susceptible Pool-16, were estimated under field conditions from June to September, 2010. Plants at 2-3 leaf stage were inoculated with viruliferous leaffopper vector, *Cicadulina triangula*. Non-inoculated plants of the same, protected by spraying at weekly intervals with lambdacyhalothrin oil served, as control. The inoculated plants of each entry were compared with respective uninoculated entry. MSV incidence was 100% in infected trial irrespective of the genotype, but severity differed significantly (p < 0.01). Plants of protected trial remained disease free. Area under the disease progress curve (AUDPC) indicated that 52.9% of the entire genotypes were moderately resistant and the remaining were moderately susceptible to MSV. In all, 75% of the moderately resistant hybrids recorded AUDPC value higher than the Tzi3. In contrast, AUDPC value of moderately susceptible hybrids were less than Pool-16. There were substantial differences among the entries in the extent of plant growth and yield parameters relative to the corresponding uninfected controls. MSV infection in the hybrids reduced plant height (7.9 to 31.6%), cob weight per plant (12.5 to 52.1%), grain weight per plant (15.4 to 58.8%), 100-kernel weight (3.6 to 29.4%) and kernel number per plant (13.8 to 61%). These results suggests that cultivation of high-yielding genotypes with adequate protection against streak disease would contribute to increase in maize productivity.

**PGPR mediated IPM for tropical vegetables in South India**

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In IPM for major tropical vegetable crops (tomato, eggplant, okra and onion) in South India, PGPR (plant growth promoting rhizobacteria) viz., *Pseudomonas* fluorescens and *Bacillus* sp were used as one of the IPM components for managing pests and diseases. The *Pseudomonas* fluorescens (TNAU-PF 1) biopesticide formulation as seed treatment (10 g/kg) followed by soil application (2.5 kg/ha) significantly reduced the incidence of major insect pests (both sucking and chewing insects) and damage caused by plant pathogens (fungi, virus and nematodes) occurring in major tropical vegetable viz., tomato, eggplant, okra, onion and chilies in various laboratory and field studies conducted. The major mechanism involved in *Pseudomonas* fluorescens (TNAU-PF 1) biopesticide mediated IPM resistance (ISR) in host plants. The ISR activity in plants was associated with increased in activity of defense related proteins viz., chitinase, glucanase, peroxidase and polyphenol oxidase. Also, the population of natural enemies (coccinellicids and spiders) of insect pests was increased. In all the field trials with PGPR as one of the IPM components, the yield was significantly increased and good market quality of vegetable products was obtained.

**New host record for *Pseudomonas syringae* on *Lomatium* spp.**

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Phytopathology 101:S159

A leaf spot disease of *Lomatium* spp. (*L. trierinatum, L. grayi* and *L. dissectum*) was observed during summer 2009 and 2010 in an experimental plot at Malheur Experimental Station, Ontario, OR. Symptoms on leaves initiated as water-soaked or translucent lesions that soon expanded and turned brown to black. Infection was severe under arid [hot and dry] conditions resulting in leaf collapse or a blighting effect. The disease was randomly distributed throughout the plots affecting *L. trierinatum* (25% plants) followed by *L. dissectum* (5–8%) and *L. grayi* (5%). Microscopic examination of the necrotic tissues revealed characteristic bacterial streaming and isolations consistently yielded a yellow-pigmented strain isolated from all the hosts. Pathogenicity was confirmed by spray inoculations on to healthy leaves of *L. trierinatum*. Typical symptoms developed on spray inoculated cilantro (*Coriandrum sativum*) but not on celery (*Petroselinum crispum*). The bacterium was identified as *Pseudomonas syringae*, based on carbon source utilization (Biolog, Inc., Hayward, CA) and MIDI microbial Identification System (Microbial ID, Inc., Newark DE) although pathovar conclusions differed and similarity coefficients were variable. To our knowledge, this is the first report of occurrence of *P. syringae* on *Lomatium* spp.

**Overseas migration affects the status of insecticide resistance in domestic populations of the small brown planthopper, *Laodelphax striatellus***

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The small brown planthopper (SBPH), *Laodelphax striatellus*, in one of the serious pests of rice plants in many Asian countries. A large overseas migration of SBPH was reported in western parts of Japan in June 2008. The immigrant populations were resistant to imidacloprid but not fipronil, while domestic ones were resistant to fipronil but not imidacloprid. Insecticide resistance to imidacloprid and fipronil was compared among local populations in western regions in Japan after the overseas migration. In some populations collected near west coast, the resistance status coincided with that of the immigrant populations just after migration, i.e., resistance to imidacloprid but susceptibility to fipronil. In other populations collected relatively far from the west coast, resistance was observed against not only imidacloprid but also fipronil. It is likely that the status of the latter populations resulted from intercrossing between immigrant and domestic populations. The resistance status in each of populations had been maintained until the next spring after over wintering. Insecticide resistance was also assessed in other areas of northern and eastern parts of Japan. In general, these populations showed relatively low resistance, although resistance to fipronil was high in the eastern part of Japan where the density of domestic populations has recently increased.

**Effect of fungicide and plant defense activator drench applications for controlling Fusarium wilt of watermelon**

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Fusarium wilt (FW), caused by fungus *Fusarium oxysporum f. sp. niveum* (FON), is a soil borne disease of watermelon that causes significant losses to U.S. watermelon growers every year. Seven years is the recommended crop rotation for fields where FON is severe, but lengthy rotations are not economically feasible for most growers and alternatives to crop rotation are needed. In this investigation, fungicide drenches and one plant defense activator drench were tested for the control of FW in a field trial at the Vidalia Onion and Vegetable Research and Educational Center in Lyons, Georgia. Plots (30 ft × 6 ft) were replicated six times in a randomized complete block design. The soil was inoculated prior to transplanting by applying 50 ml of a 1 × 10^6 microconidial suspension of FON race 1 to holes that watermelon transplants (cv ‘Black Diamond’ were planted into. Treatments were applied after watermelon were transplanted by drenching each plant with either a fungicide solution, a solution of the plant defense activator or fungicide + plant defense activator + water control. One foliar application of each treatment was applied one month after planting. Disease incidence was assessed during the season. Treats treated with Actigard demonstrated significantly less FW incidence than the untreated control, and there was no significant difference between Actigard treated plots and plots treated with Proline (prothioconazole) or V1016 (metconazole).

**Mycelial growth and sporangial production of *Phytophthora capsici* as affected by extracts from pecan tissues**

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Pecan (*Carya illinoinensis*) is an economically important nut crop in New Mexico and other regions in the U.S. Production of pecan generates several by-products including leaves, husks, shell, and wood. Previous work has demonstrated that various pecan tissues contain fungitoxic substances. Therefore, pecan by-products may be used in the control of plant pathogens. This study was conducted to examine the effects of pecan tissue extract on mycelial growth and sporangial production by *Phytophthora capsici*, a major oomycete pathogen of various vegetable crops in New Mexico and other regions within and outside the U.S. Aqueous extracts (5 and 10%, w/v) were prepared from ground tissue of leaf, husk, shell, and woody branches. *Phytophthora capsici* isolates from 7-day-old V8 culture of an isolate of *P. capsici* were placed in 25 ml extract in 9-cm diameter petri dish, and incubated in a growth chamber at 26°C under continuous light. Plugs serving as control were placed in sterile distilled water. After 48 h, plugs were examined for mycelial growth and sporangial production. The greatest mycelial growth was recorded in extract of woody tissue. Mycelial growth was lowest in shell extract. No sporangia were formed in any of the extracts from pecan tissue. Abundant production of sporangia was observed on plugs incubated in distilled water. These results suggest that extracts from pecan tissue may reduce sporangial inoculum potential of *P. capsici*.

**Genetic characterization of *Rhizoctonia solani* population isolated from sugar beet and dry bean diseases**

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Phytopathology 101:S159
Rhizoctonia solani causes Rhizoctonia root rot in sugar beet and dry bean, and crown rot in sugar beet. The diseases can cause up to 50% yield loss in both crops. Genetic diversity analysis in plant pathogen populations is necessary to understand co-evolution in plant pathosystems. The objectives of the present study were (a) to elucidate the extent of genetic variability present in R. solani isolates from the diseased sugar beet and dry bean fields in western Nebraska; (b) to determine the similarity/diversity among the isolates of sugar beet and dry bean. Twenty-eight and nine isolates of sugar beet and dry bean, respectively, were evaluated based on morphological characteristics and PCR-based DNA markers (ISSR, RAPD, and AFLP). Fungal colony colors growing in culture were variable and consisted of light tan, tan, dark tan, light brown, brown, and cream isolates. A high degree of genetic diversity among the isolates was observed based on DNA marker analysis using five ISSR primers. Total numbers of amplified bands varied from 1-20 within the range of 200 bp to 3 kb. We did not find any distinguishing DNA marker patterns between isolates of sugar beet and dry bean based on these five markers. All these isolates will be analyzed with about 50 different markers. The polymorphic DNA markers will be scored for each isolate and the data will be used for cluster analysis. This information may help in disease management and molecular pathotyping of the pathogen.

Detection of Tomato ringspot virus in rose and almond in Fars Province of Iran

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Phytopathology 101:S160

Field surveys were conducted to assess the incidence of Tomato ringspot virus (ToRSV, genus Nepovirus, Family Secoviridae) in rose (Rosa chinensis L.) and almond (Prunus amygdalus L.) in Fars Province of Iran during 2009-2010. A total of 100 leaf samples with viral disease symptoms were collected and analyzed byDouble Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) and Dot-Blot Assay (DBIA) for the presence of Tomato ringspot virus (ToRSV) with polyclonal antibodies. Serological diagnoses were confirmed by Immuno Electron Microscopy (IEM) and bioassay. Results indicated that ToRSV was present in all surveyed gardens. Among the samples tested, ToRSV was found in 21% of roses and 10% of almond trees. By applying RT-PCR to ToRSV-infected rose and almond plants, the expected 330 bp DNA fragment for ToRSV was obtained from all the samples tested. To our knowledge, this is the first report of ToRSV infecting rose and almond plants in Iran.

Effect of microbial diversity on soil fungistasis, disease suppression and colonization by biological control agents

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Biodiversity strongly affects ecosystem functions such as productivity, stability and invasibility. Research efforts have been mostly focussed on terrestrial plant diversity, while little is known about soil microbial communities. This work aims to investigate the effects of microbial diversity on soil fungistasis, disease suppression and capability of non native microbes to colonize rhizosphere. Synthetic microbial communities with species richness ranging from 1 to 8 were used in factorial experiments. Fungistasis was assessed by germination tests on various fungi (Aspergillus niger, Botrytis cinerea, Trichoderma harzianum), while disease suppression was assessed by germinating P. penetrans on soil amended with tebugban. Flowering. For all treatment combinations, the population of OH 182.9 C showed a high degree of genetic diversity among the isolates will be analyzed with about 50 different markers. The polymorphic DNA markers will be scored for each isolate and the data will be used for cluster analysis. This information may help in disease management and molecular pathotyping of the pathogen.

Antagonist Cryptococcus flavescens OH 182.9 3C colonization of wheat heads when applied with triazole fungicides and the effect on scab

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Phytopathology 101:S160

Integrated pest management (IPM) is the best available approach for reducing Fusarium head blight (FHB) and the mycotoxin deoxynivalenol (DON) in grain. Utilizing the effective FHB biological control agent Cryptococcus flavescens OH 182.9 (NRRL Y-30216) as part of an IPM approach against FHB is understudied. Trizole fungicides such as prothioconazole (PTC) used alone or in combination or in combination with prothioconazole (Prosaro) are effective against FHB, but their use generally is not recommended after wheat anthesis to control late infections. A PTC-tolerant variant of OH 182.9 (OH 182.9 3C) in a tank mix with a fungicide or applied after flowering, could reduce DON by establishing populations that reduce late DON-producing infections by Fusarium graminearum. In a two year study, the colonization of gluten and yellow grain was better by OH 182.9 3C was determined when the agent was applied alone or in combination with a fungicide at or seven days after wheat flowering. For all treatment combinations, the combination of OH 182.9 3C represented 50–95% of the total microbial population recovered from both gluten and endosperm from 8 to 11 days after flowering, demonstrating the competitive success of the strain. While the application of strain OH 182.9 3C at times reduced (P < 0.05, FPLSD) FHB and/or DON, combinations of fungicide and antagonist were rarely significantly more effective than either component used alone for the doses tested.

Characterization of Cylindrocarpon populations associated with replant disease of almond and peach

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Phytopathology 101:S160

Growth and cumulative yield of replanted almond and peach orchards are often seriously compromised by Prunus replant disease (PRD), a poorly understood soilborne complex affecting successive plantings of Prunus. Previously, our culture-based (CB) and culture-independent (CI) examinations of fungal, bacterial, and nematode communities revealed that the fungus Cylindrocarpon (Cyl) was among organisms commonly associated with PRD, yet little is known of Cyl on Prunus. We examined these Cyl species using CB and CI methods. Eighty-eight cultured isolates, each from roots of a different
tree, were obtained from six California (CA) almond and peach orchards affected by PRD. The isolates were identified based on BLASTn searches of sequences of three loci (rDNA ITS, beta-tubulin, mtSSU rDNA). Also, from a subset of 17 of the trees among three of the six orchards, Cyl populations were examined using CI amplification and sequencing of ITS 2 DNA fragments. Neighbor-joining cluster analysis and BLAST searches identified 87 of the cultured isolates as C. macrodidymum; one was C. liriodendri. Similarly, 117 of the 121 Cyl clones were C. macrodidymum; four were C. destructans. Our results indicate that C. macrodidymum is the most prevalent species of Cyl associated with PRD in CA. We are testing pathogenicity and aggressiveness of Cyl on Nemaguard rootstock for almond and peach; preliminary results indicate that at least some isolates of C. macrodidymum are pathogenic.

High planting combined with root collar excavation extends life of peach trees on Armillaria root rot infested replant sites

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Phytopathology 101:S161

Armillaria root rot is a serious disease of peach, killing thousands of trees every year in the Southeastern United States. In this study we investigated the survival of peach trees planted in 45 × 60 cm open-bottom SmartPots about 40 cm higher than the grower standard on two Armillaria root rot infested replant sites. The root collars were excavated after one growing season on half of the potted trees. After five years, almost all trees with excavated roots were still alive and productive at both locations, whereas 60% and 20% of control trees had died in locations 1 and 2, respectively. Trees left in pots for the entire 5 years were found to be more resistant to Armillaria root rot compared to the grower standard. Across years root suckering of rootstock was no different between treatments, however, potted trees were more susceptible to drought in the absence of irrigation the year of establishment. The results show that root collar excavation of peach trees planted high may be a suitable approach to lengthen the life span of trees on replant sites with high Armillaria root rot pressure. Research is underway to investigate commercialization of this system in commercial peach production areas.

RIFdb: An online database for the classification of plant-associated bacteria using the computationally derived RIF marker

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Phytopathology 101:S161

A DNA marker that distinguishes plant associated bacteria at the species level and below was derived using comparative genomics of six sequenced genomes of Xanthomonas, a genus that contains many important phytopathogens. This DNA marker comprises a portion of the dnaA replication initiation factor (RIF). Unlike the rRNA genes, dnaA is a single copy gene in 95.2% of the sequenced bacterial genomes, and amplification of RIF requires genus-specific primers. The RIF DNA marker was sequenced using genus-specific primers for 315 Xanthomonas, 210Ralstonia, 11 Pectobacterium, 6 Pantoea, 7 Dickeya and 114 Clavibacter characterized strains in the Pacific Bacterial Collection and 43 Xanthomonas strains from the International Collection of Microorganisms from Plants. Genus-specific primers were also developed for Xylella and Pseudomonas, and RIF sequences were extracted from the sequenced genomes of five and twenty-three strains. Inter-RIF alignments were available at RIFdb, and can be queried in both chromatogram and FASTA format with RIF sequences obtained from unknown strains. This database provides an easy access point to help classifiers classify plant-associated bacteria and compare local strains with a worldwide collection of phytopathogenic bacteria.

Effects of Bacillus firmus GB-126 on the Soybean Cyst Nematode mobility in vitro

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Phytopathology 101:S161

Cells and cell-free extracts of Bacillus firmus strain GB-126 were evaluated for their capacity to reduce mobility and cause paralysis of juveniles of the Soybean Cyst Nematode (SCN) (Heterodera glycines). Two in vitro assays were conducted to measure mobility and paralysis of J-2s of SCN and G. Necrotrophs. The experiments were conducted in a 96-well plate format containing 100 µl of GB-126 cells at 1 × 10⁶ and 1 × 10⁷ cfu/ml and cell-free extracts at 100%, 50%, and 25% concentrations. Juveniles were evaluated for mobility and paralysis with a Nikon TS100 inverted microscope at 0, 12, 24, and 48 hours. GB-126 cells at both concentrations significantly reduced mobility compared to Tryptic Soy Broth (TSB) and Sterilized Tap Water (STW) controls at 36 h after treatment. Mobility was reduced to 61% and 67%, respectively, in the 1 × 10⁶ and 1 × 10⁷ cfu/ml cell suspensions at 48 h of exposure. With cell-free extracts, mobility was significantly reduced 12 h after treatment with 100% and 50% concentrations compared to TSB and STW controls. Mobility of SCN J2s ceased completely to a paralytic form in the 100% cell-free extract concentration at 48 h. The 50% and 25% cell-free extract concentrations reduced mobility of SCN J2s by 95% and 54%, respectively, at 48 h. The results of the experiments indicate that both cells and cell-free extracts of GB-126 can have direct effect on SCN J2.

Survey of Rhizoctonia spp. from wheat soils in the U.S. and determination of pathogenicity on wheat and barley

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Rhizoctonia root rot and bare patch are chronic diseases of wheat and barley in the Pacific Northwest (PNW), but little is known about Rhizoctonia spp. in other cereal growing areas of the U.S. A survey was conducted in the fall of 2009 and 2010 to identify Rhizoctonia spp. from soils collected throughout the wheat growing regions of the U.S. Soils were collected from 114 fields in 14 states and Rhizoctonia isolates were bailed from these soils using a toothpick baiting method. Recovered isolates were identified by sequencing the ITS region of the rDNA and comparison of the sequence with the sequence of previously identified reference strains. Isolates were recovered from 49 locations and 51 isolates were sequenced. Rhizoctonia solani AG-2-1 (27%) and R. oryzae (Waitea circinata) (39%) were the most common species found. Rhizoctonia solani AG-3, AG-4, AG-10 and AG-11; and Ceratobasidium sp. AG-A and AG-I were also found. Interestingly, R. solani AG-8 was not found outside of the PNW. In pathogenicity assays conducted in the greenhouse in pasteurized soil, most isolates caused significant plant stunting and displayed typical root disease symptoms compared to the non-inoculated control. The highest disease ratings (0-8 scale) were observed with AG-3 (2.2 on wheat, 2.7 on barley), AG-A (1.9 on wheat, 2.8 on barley), and AG-4 (1.7 on wheat, 2.1 on barley). This data suggests that other groups of Rhizoctonia may be capable of causing damage on wheat and barley.

Overview of the Onion ipmPIPE and the development of innovative disease diagnostic tools for onion diseases


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The overall goal of the Onion ipmPIPE Project is to incorporate existing onion pest management programs and pest risk assessment models developed at local and regional levels into an internet platform for national implementation and validation. This project will develop a systems based trans-disciplinary approach to onion production to improve the productivity and profitability for onion stakeholders. The Onion ipmPIPE Project will expand the innovative diagnostic tools and coverage for priority diseases of onion caused by fungal and bacterial pathogens and their complexes. The Onion ipmPIPE will link with weather impact management tools and recommendation systems. IYSV and thrips are among the top priorities for the Onion ipmPIPE component which will be monitored via sentinel plots. In addition, the complexes of bacterial and fungal diseases assume an important secondary priority in terms of developing monitoring and sampling protocols. A set of diagnostic cards have been developed to facilitate rapid and accurate visual identification of onion growth stages, diseases and pests. The national expert laboratories are working in the process of developing high throughput molecular methods to aid in the diagnosis of diseases caused by fungal and bacterial pathogens. Educational on-line resources such as the expanded Alliumnet and Pest Image Gallery and Bugwood Wiki hosted by CISEH Bugwood on an Onion ipmPIPE infrastructure will be accessible via the Web.

Legume ipmPIPE—A real-time disease/pest monitoring and reporting network


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The Legume ipmPIPE objectives and accomplishments comprise a variety of programs and resources for common beans, cool-season legumes and warm-season legumes including: (a) established sentinel or mobile plots in as many as 25 states in the United States, Canada and Mexico through collaborations with in-country scientists; (b) identified priority diseases and pests for monitoring; (c) monitored fungal and bacterial diseases and insect pests throughout the growing season; (d) implemented sampling protocols to monitor viral disease prevalence and kit-based high output immunosassays for six common legume viruses for use by National Plant Diagnostic Network labs; (e) established communications between scientists specializing in legumes across the U.S.; (f) collected and archived data from across the U.S.; (g) supported a web-based platform for access and information display to extension educators, researchers, extension agents, industry, and other stakeholders; (h) created a web-based portfolio of management and education tools; and (i) distributed a popular series of pocket sized cards (print and online versions) that improve the accuracy of pest and disease diagnostics. The ultimate goal of the Legume ipmPIPE remains identifying causes of loss in legumes and assisting producers in minimizing those losses by implementing timely and economical components of Integrated Pest Management for priority pathogens and pests.

Pyrethrum yield estimation by digital image analysis

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Phytopathology 101:S162

Pyrethrum is grown for the production of insecticidal pyrethrins. Production in Australia is constrained by a number of fungal pathogens, including Stagonospora ligulicola and Stagonospora laevispora. To accurately assess control strategies for these and other diseases, a practical method of yield assessment is required. Previously, yield assessment has relied upon destructive, manual harvesting, which is both costly and time consuming. As an alternative, yield estimation by digital image analysis was trialled at 29 sites in summer 2010, with replication. Images were taken at a height of 0.9 m above the crop canopy, encompassing a defined 0.7 × 0.7 m quadrat, placed level with flower height. Image capture occurred once over 50% ray florets were open within a crop. With the software package ImageJ, HSB color thresholding was used to isolate the yellow centres of individual flowers, from which the particle analyser function estimated the number of flowers per quadrat. Linear regression indicated a strong relationship between automated (AC) and manually measured flower counts, (AC = 21.1 + 0.97*MC; R² = 0.95, P < 0.0001) with an intercept not significantly greater than zero. A significant correlation was also obtained between automated flower counts and hand harvested dry weight of flowers (DW) from each quadrat (AC = 109.1 + 3.45*DW; R² = 0.488, P < 0.0001). These results highlight the potential for predicting yield using non-destructive image capture.

Disease incidence and race characterization of Fusarium wilt

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Phytopathology 101:S162

Cotton cultivars were examined for susceptibility to Fusarium wilt (FW); subsequently fungal isolates were collected to confirm Fusarium oxysporum f. sp. vasinfectum (FOV) presence and race. Symptoms of FW were initially observed June 23 on Stoneville 4288B2RF. By July 7, Deltapine 9949B2RF, DP1050B2RF, DP1028B2RF, Phytoget 375WRF, PHY485WRF, PHY565WRF and the susceptible Rowden were symptomatic. FiberMax 1740B2RF developed symptoms two weeks later. By Aug. 26, FW incidence culminated with 16% of the Rowden and <1% of the resistant control M-315 plants dying. FOV was re-isolated on APDA from hypocotyls regions of all cotton cultivars except PHY367WRF, ST545B2RF, and M-315. Morphologically, FOV colonies were white, loosely floccose with reverse pigmented, cream to tan, fibrous, ring forming over time. Phialides were monophialidic, short and single. Microconidia were abundant ellipsoidal, 1 celled, averaging 10.09 ± 2.58 µm. Macroconidia were falcate with tapering apical and basilar, 3-5 septate measuring an average of 18.40 ± 4.86 µm. Chlamydospores were rough-walled, sub-globose, and 8.73 ± 3.4 µm in diam. Genetic analysis of each FOV isolate, by variety, was characterized by partial sequences of the EF-1α gene, indicating very diverse clades of genotypes within the field. Combined analysis done in CA exposed genotype races 1, 2, 4, 8, and several undefined genotypes to be present. Results of this field test indicate resistance to FW does exist in our cotton cultivars, and FOV in Alabama appears to be extremely diverse genetically.

Integrated management of invasive mealybugs in brinjal

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Phytopathology 101:S162

Brinjal is widely grown in Tamil Nadu, India. Apart from shoot and fruitborer, mealybugs are the major concerns for farmers. Cocidoxystrux insolita is the mealybug which occurs in the late stage of the crop growth. Two invasive mealybugs viz., Selenopseus mealybug, Phenacoccus selenopseus and papaya mealybug, Paracoccus marginatus were found heavily damaging the brinjal. Integrated management practices involving timely monitoring, the use of non-affected plant parts, and weeds, conservation of predacious coccinellid and parasitoids and need based insecticides were recommended. Development of resistant variety will give a long term real solution in combination with parasitoids and natural enemies. Wild Solanum viarum is found to be free from damage by both the mealybugs and the mechanism of resistance is studied with cheap method of mass multiplication of Harmonia axyridis, plants and parasitoids. The non preference mechanism of resistance was exhibited in S. viarum high trichome length and density, more thickness of leaf and phloem region. Laboratory studies indicated that prophenofos, dimethoate Pseudomonas fluorescens and Beauveria bassiana showed better ovicidal action against P. marginatus causing high per centage of egg mortality.

Characterization of Phytophthora infestans from Wisconsin in 2009 and 2010

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Phytopathology 101:S162

Late blight, caused by the oomycete Phytophthora infestans is the most limiting disease to potato production worldwide. After 2002, Wisconsin growers enjoyed a 6-year respite from this disease, until it appeared in 2009 and 2010. In these years, 33 isolates were collected from potato and tomato from across the state. Allozyme genotype was resolved using cellulose acetate electrophoresis at the Glucose-6-phosphate isomerase locus. This revealed 3 banding patterns: 100/122 (US-22), 100/US-23, and 100/100/111 (US-24). Sensitivity to the fungicide mefenoxam was determined by measuring percent radial growth on Rye A media containing 100 ppm mefenoxam compared to an unamended control. US-22 and US-23 showed sensitivity, averaging 6.7 and 14.3% growth respectively, while US-24 showed partial insensitivity, averaging 42.4% of the control. This indicates that use of allozyme genotyping can aid in the selection of mefenoxam to control late blight. Mating type was determined by plating each isolate with a known A1 (US-1) and A2 (US-7) on Rye A and observing the presence of oospores. US-22 formed oospores with US-1, indicating A2 mating type, and US-23 and US-24 formed isolated oospores with US-7, indicating A1 mating type. Isolates of opposite mating types were geographically isolated in the state, but potential exists for oospore formation in subsequent years, which could challenge current late blight management strategies.

Effects of temperature on potato zebra chip disease development

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Phytopathology 101:S162

Temperature has been shown to have significant impact on development of liberibacter species associated with citrus Huanglongbing disease. "Candidatus Liberibacter africanus" and "Ca. L. americanus" are both heat sensitive, whereas "Ca. L. asiaticus" is heat tolerant. The recently described "Ca. L. solanacearum" is associated with zebra chip (ZC), a newly emerging and economically important disease of potato worldwide. This psyllid-transmitted liberibacter species severely affects several other solanaceous crops and carrot. Experiments were conducted to evaluate effects of temperature on development of "Ca. L. solanacearum" and ZC disease. Potato plants were maintained at selected temperature regimes in growth chambers and monitored for ZC symptom development and later tested for liberibacter by polymerase chain reaction to confirm infection. Results indicated that temperatures below 17°C appear to slow development of "Ca. L. solanacearum" and ZC symptoms whereas temperatures above 35°C are detrimental to this liberibacter. Compared to Huanglongbing Liberibacter, "Ca. L. solanacearum" appears heat tolerant. This sensitivity of this bacterium and its insect vector to temperature may partially explain incidence, severity, and distribution of ZC in affected regions.

Epiphytic yeasts for biocontrol of Botrytis cinerea on table grapes cv. Thompson seedless

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Phytopathology 101:S162
Botrytis cinerea, the causal agent of gray mould disease, causes severe economic losses on postharvest fruit and vegetables, particularly on table grapes in Chile. Traditionally, gray mould control has been based on application of fungicides. However, the growing public concern about fungicide residues in food and environmental risks associated with its use, and the development of resistant strains of these fungal pathogens have generated interest in the development of alternative, biological control methods. Yeasts. (N = 125) isolated from grape and apple surface fruits were tested in a preliminary screening on agar plates, of which 8 inhibited the growth of mycelium of B. cinerea, in grade 2 to 4 on the scale proposed by Swadding and Jeffries (1996). Antagonist activity of these strains was also evaluated at different pH (4.2, 4.6, 5.0 and 5.4), resulting the pH 4.2 the most favorable. Two yeast isolates-chemical methods of control, like biological control. Yeasts. (N = 125) isolated from grape and apple surface fruits were tested in a preliminary screening on agar plates, of which 8 inhibited the growth of mycelium of B. cinerea, in grade 2 to 4 on the scale proposed by Swadding and Jeffries (1996). Antagonist activity of these strains was also evaluated at different pH (4.2, 4.6, 5.0 and 5.4), resulting the pH 4.2 the most favorable. Two yeast isolates-chemical methods of control, like biological control.

Establishment of a TMV-based transient expression system for AMPs in plants and their in planta/in vitro activity against compatible pathogens K. H. SHAH (1), H. Bohlmann (1) (1) Institute of Plant Protection (IFP), University of Natural Resources and Life Sciences, Vienna, AUSTRIA Phytopathology 101:S163 There have been discovered several antimicrobial peptides (AMPs) in many plants and animals secreted in response to various pathogens via innate immune system. These include variety of relatively small, basic, cysteine rich peptides such as Defensins, Thionins, Lipid Transfer Proteins and Snakins. These AMPs have shown antimicrobial activity in vitro and some of them have been expressed in transgenic plants leading to higher resistance against different pathogens. Previously it was found that Arabidopsis contains hundreds of genes that potentially encode putative AMPs such as thionin like peptides. We have developed a TMV RNA based efficient transient expression system (pPZP5000) to express these peptides in Nicotiana benthamiana leaves by agroinfiltration. In a comparison assay of five different plasmid vectors by expressing markers (GFP and GUS), pPZP5000 came out as the best system for the expression of up to hundreds of genes in plants therefore, we are using it to produce putative AMPs to test their activity in two ways: First, we will isolate the peptides for in vitro tests against bacteria and fungi. Second, activity will be tested in planta by infecting leaves that transiently express peptides with compatible pathogens.

First report of apple canker caused by Xanthomonas sp. from Iran N. SHAJ (1), N. Hasanzadeh (2), E. Nazerian (3), M. Keshavarsi (4) (1) Azad University of Tehran, Tehran, IRAN; (2) Islamic Azad University, Science and research branch, Tehran, IRAN; (3) Plant Protection Department, Faculty of Agriculture, University Putra Malaysia, Serdang, MALAYSIA; (4) Seed and Plant Improvement Institute, Karaj, IRAN Phytopathology 101:S163 A local apple cultivator showing canker symptoms in one commercial orchard of northern Iran were sampled and brought to the laboratory during spring and summer in 2010. Disease symptoms were gray to dark brown discoloration with the expanding oozing. A total of 50 samples were collected. Small pieces of twigs and branches were emersed in 5 ml of saline solution (0.80% NaCl) for 20 min to disperse the bacterial cells. Thirty micro liters of the resulting suspension was separated on nutrient agar (NA) medium and incubated at 30°C for three days. Purification of cultures were repeated twice on this medium. Biochemical and physiological test carried out according to standard method. Hyperactivation reaction with infiltration of 100 CFU/ml of bacterial suspension in to the Geranium leaf epidermis was positive. In pathogenicity test detached fresh apple shoots were inoculated with needle charged of 108 CFU/ml of the representative bacterial suspension. Inoculated and uninoculated (control) samples were placed in growth chamber with 80–90% relative humidity at 27°C. Symptoms that occurred 4 days after inoculation were the same as naturally infection, while samples inoculated with water as control, remained healthy. Based on biochemical, physiological and PCR amplification with RS21/RS22 primers all isolates were identified as Xanthomonas sp. This is the first report of apple canker caused by Xanthomonas from Iran.

A multiplex RT-PCR for detection of three Cucurbitis-infecting poleroviruses Q. SHANG (1), H. Xiang (2), C. Han (2), D. Li (2), J. Yu (2) (1) Beijing University of Agriculture, Beijing, PRC PEOPLES REP OF CHINA; (2) China Agricultural University, Beijing, PRC PEOPLES REP OF CHINA Phytopathology 101:S163
The presence of three poleroviruses including Cucurbit aphid-borne yellows virus (CABYV), Melon aphid-borne yellows virus (MABYV) and Suakwa aphid-borne yellows virus (SABYV) in India were definitely confirmed in our preliminary surveys. Here we reported a multiplex RT-PCR method for rapid detection and differentiation of these three Cucurbitis-infecting poleroviruses. The multiplex PCR assay was developed with a mixture of universal primer P0cocokp and species-specific primer sets CA3414F, MA3639F and SA3133F selected to differentiate three viruses CABYV, MABYV and SABYV, respectively. The mRT-PCR products consisted of fragments of 700bp for CABYV, 450bp for MABYV and 950bp for SABYV. Amplification of three target viruses was optimized by increasing the PCR annealing temperatures. And, the sensitivity of the multiplex PCR detection for CABYV, MABYV and SABYV were also evaluated in the study. Detection limit for PCR products was 1pg for CABYV, 0.1pg for MABYV and 1pg for SABYV. The multiplex RT-PCR is found a specific, sensitive and cost-effective method to detect multiple poleroviruses in cucurbits. This detection technique may facilitate research on cucurbits-infecting poleroviruses epidemiology, outbreak monitor and host-virus interaction analysis. The research is supported by the fund from National Natural Science Foundation of China (31000840), the Natural Science Foundation of Beijing City (6082006) and the Science and Technology Nova Program of Beijing (2007B032).

Pathogenic and genetic diversity in Alternaria brassicola and Alternaria brassicicola causing black leaf spot of cauliflower in India

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Phytopathology 101:S164

Biological pathotyping of Alternaria brassicola (21) and Alternaria brassicicola (6) isolates collected from cauliflower grown in seven different states of India were evaluated against a set of six cauliflower cultivars under lab and greenhouse conditions. Based on the pathogenic reaction, following seven groups were formed: Pathotype I (all cultivars); Pathotype II (all except Pusa Kartik Shankar); Pathotype III (all except Pusa Meghana); Pathotype IV (all except Pusa Sharad and DC-41-5); Pathotype V (all except Pusa Deepali and Pusa Kartik Shankar); Pathotype VI (Pusa Meghana and DC-23000); Pathotype VII (only Pusa Meghana). Based on disease severity ten isolates of Alternaria brassicola were Less aggressive, eight Moderately aggressive and three including six of A. brassicicola were Highly aggressive. Genetic analysis was performed using 42 RAPD and 3 ISSR primers. Combined dendrogram produced clearly distinguished between A. brassicola and A. brassicicola populations at 0.48 similarity levels which when related with pathotyping data formed 13 and 3 lineage groups respectively though being geographically distinct belonged to same pathotype. Internal transcribed spacer sequences (ITS) within the ribosomal DNA (rDNA) region were targeted to explore genetic variability among above Alternaria isolates. Phylogenetic relationship based on the ITS sequence and PCR-RFLP of amplified rDNA sequences clustered the A. brassicicola distinct from A. brassicola similar to RAPD and ISSR analysis irrespective of pathogenicity.

Biological control of root rots of groundnut in Rajasthan, India

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Phytopathology 101:S164

Groundnut is an important oilseed crop predominantly grown in Rajasthan, India and 85 percent root rot was caused by multiple pathogen complex mainly Aspergillus niger, Apergillus flavus, Thielaviopsis basicola, Rhizoctonia solani and Pythium aphanidermatum infecting seed, soil and crop. Biocontrol technology using Trichoderma harzianum (Th3) was used against Groundnut varieties, GG-10, GG-20 and Local varieties in 2009 and 2010 at farmer’s fields in twelve different villages of Jaipur district. Field trials on soil, seed and foliage treatment with powdered bioformulation (Th3 SD, Th3 SS, Th3 SDSS and Th3 SS) seed/soil followed by the spray treatment of the liquid bioformulation (Th3 FS) @ 4-5 ml/l along with recommended IPM practices were conducted. The untreated crops were significantly low in yields with the diagnostic blackening symptoms travelling from roots to stem affecting the vascular system followed by shedding at root-stem intermutes resulting in complete wilting and plant death while in treated crop blackening reduced. A healthy plant vascular system was free of disease. Maximum values of R.C. Index (0.15), C.F.U. (58.5 × 106), seed germination (85%), pod yield (40 Q/ha) and lowest root rot incidence (15%) was recorded. Participatory approach and interaction between researcher and farmers helped in quick adoption and dissemination of use of biocontrol agents for groundnut growers in Rajasthan state, India.

Characterization of silencing suppressor activity of Ns from Iris yellow spot virus (Genus Tospovirus)

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Iris yellow spot virus (IYSV), a tospovirus, causes serious losses to onion bulb and seed crops. The viral genome consists of three RNAs, large (L), medium (M) and small (S). The S RNA codes for a non structural protein (NSs) in sense direction which was shown to function as the viral suppressor of gene silencing in plants. To further characterize the suppressor activity of NSs, a series of constructs of various lengths of IYSV NSs gene were made in pCAMBA vector. These constructs were mobilized into Agrobacterium and plants of Nicotiana benthamiana line 16c, expressing GFP were agro-infiltrated either with or without a GFP expressing pCAMBA vector. The ability of various NSs constructs to relieve the suppression of GFP was evaluated. This approach facilitated a better understanding of the role of NSs as the silencing suppressor during tospovirus infection of plants.

Pathogenic variation in Pyricularia grisea, the causal agent of pearl millet blast and resistance in mini core collection to the pathogen

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Phytopathology 101:S164

Pearl millet blast caused by Pyricularia grisea (Cooke) Sacc. [teleomorph: Magnaporthe grisea (Herbert) Barr] has emerged as a serious disease during the past few years in several states in India. Pathogenic variation in P. grisea was studied through pearl millet blast variability nurseries (PMBVN) established at four locations (Gwalior, Anand, Dhule and Patancheru) in India during the rainy season 2010, and by seedling reaction of host differentials to P. grisea isolates in a greenhouse screen. Four genotypes (ICMR 0622, ICMR 06444, ICMR 97222 and ICMR 99944) of the 20-entry PMBVN showed differential reaction across the test locations indicating pathogenic variation in P. grisea in India. This was confirmed in a greenhouse screen by the ten pearl millet genotypes to 25 isolates of P. grisea collected from major pearl millet growing areas in India. Based on the differential reactions, 25 isolates formed ten distinct pathogenic groups/pathotypes. Sources of blast resistance were identified by evaluating 238 pearl millet mini core accessions (1% of entire collection representing most of the useful variation) against a Patancheru isolate of P. grisea under greenhouse conditions -ten accessions (IP 4291, IP 4488, IP 5964, IP 7358, IP 8913, IP 9692, IP 11044, IP 13636, IP 21503 and IP 22449) were found resistant. The mini core accessions will be further evaluated to identify resistance to multiple pathotypes.

Virulence diversity of international collections of the wheat stripe rust pathogen, Puccinia striiformis f. sp. tritici

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Virulence information in the wheat stripe rust (yellow rust, Yr) pathogen, Puccinia striiformis f. sp. tritici (Pst), is important for controlling the disease with resistant cultivars. A total of 236 Pst isolates from Algeria, Australia, Canada, Chile, China, Hungary, Kenya, Nepal, Pakistan, Russia, Spain, Turkey, and Uzbekistan were tested on 20 single-gene lines and the 20 wheat genotypes for differentiating U.S. races. Thirty U.S. isolates representing 15 major races in 2006–2009 were selected for comparison. The 236 isolates were identified as 115 races on the single-gene lines and 160 races on the U.S. differentials. None of the isolates were virulent to resistance genes Yr1 and Yr17. Virulences to Yr10, Yr24, Yr26, Yr31, and YrExp2 were widespread, those to Yr1, Yr3, Yr2, Yr3, Yr17, Yr26, Yr31, YrExp2, Yr21, Prodira (YrPr1, YrPr2), Stephens (Yr3a, Ys, YrSte), Lee (Yr7, Yr22, Yr23) and Fielder (Yr6, Yr20) were high (80%); and those to Yr1, Yr8, Yr9, Yr25, Yr27, Yr28, Heines VII (Yr2, YrHV11), Paha (YrPa1, YrPa2, YrPa3), Druchamp (Yr3a, YrD, YrDru), Yamhill (Y2, Yr4a, YrTum), Tye (YrTy), Trees (YrTr1, YrTr2), Hyak (Yr17, YrTye), Express (YrExp1, YrExp2), Clement (YrClem) and Compar (YrCom) were moderately frequent (20–80%). Although races were generally different, most of the virulences were common in these countries. The virulence data indicated gene flow between some of the countries.
Effects of DMI fungicide applications on secondary metabolites in creeping bentgrass (Agrostis stolonifera L.)

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Geranylgeranyl diphosphate is the 20-carbon compound in the isoprenoid pathway that gives rise to the diterpene gibberellin (GA) compounds and the tetraterpene carotenoid pigments. Applications of DMI fungicides inhibit GA synthesis in plants blocking the GA pathway, we hypothesize that a shift of metabolic precursors from normal GA synthesis to the carotenoid pathway may occur. Carotenoids act as powerful antioxidants in plants; an increase in products of the carotenoid pathway, particularly the xanthophyll cycle, may result in improved stress tolerance. The objective of this study was to determine carotenoid and chlorophyll (chl) concentrations in creeping bentgrass following applications of propiconazole (PPZ) and tebuconazole (TBZ). Two applications of PPZ and TBZ were made at 7 day intervals at rates of 0, 976, and 1952 g a.i. ha⁻¹. Leaf blades were harvested 7 days after the last treatment. Carotenoid and chl pigments were extracted from frozen leaf blades and measured using HPLC. Violaxanthin concentrations increased approximately 20–25% while zeaxanthin concentrations dropped 21–28% compared to non-treated plants. Both fungicides significantly increased chl a, chl b, and total chl. Fluxes in xanthophyll pigments towards increased violaxanthin concentrations indicate that the plant may tolerate stresses better than non-treated plants. Further research is needed to determine if changes in xanthophyll pigments result in improved quality under stressful conditions.

QTL mapping of resistance genes for eyespot of wheat in Aegilops longissima

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Eyespot is an economically important disease of wheat caused by the soilborne fungi Oculumacula� yallundae and O. acuformis. Resistant cultivars are the most desirable control method, but resistance genes are limited in the wheat gene pool. A wild wheat, Aegilops longissima (2n = 14, SS′), was evaluated as a new source of resistance to eyespot. A recombinant inbred line (RIL) population developed from the cross PI 542196 (R) x PI 330486 (S) was used to construct genetic linkage map of the S l genome with 169 wheat SSR markers covering 1261.3 cM in 7 groups. F 5 lines (189) were tested for resistance. Concordance between GUS scores and visual disease rating. These results demonstrate that genetic control of resistance genes to wheat and broaden the genetic diversity of eyespot resistance.

Integrating Sedaxane as part of a comprehensive seed care product for broad spectrum disease protection of small grains

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Seed and soil borne diseases have constantly challenged cereal small grain plant establishment and the consequent growth and development. These problems are also compounded when small grains are planted in no–till or low till conditions. Shifting trends of rotational crops in traditional small grain production areas have also increased the diversity and complexity of pathogens that affect grain seedlings. Seed Care solutions are now integral to overall crop protection strategies. The integration of Sedaxane as a new mode of action to existing seed care products expands the broad spectrum and sustainable management of seed applied fungicides. In wheat soil survey new strains of Rhizoctonia sp. have been identified and the role of Sedaxane is evident. In addition Sedaxane seed treatment has also shown excellent activity against Ustilago nuda in barley. Field and greenhouse studies suggest that Sedaxane offers a unique advantage in preserving root health against constant challenge of seed and soil borne pathogens.

Biological characteristics regulated by algU in Xylella fastidiosa

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Xylella fastidiosa is a plant xylem-limited and Gram-negative bacterium that causes Pierce’s disease in grapes through cell aggregation and vascular clogging. algU encodes an alternate sigma factor, AlgU, that confers tolerance to osmotic, oxidative, and the transcriptional regulation of function of pili in gram-negative bacteria including Pseudomonas aeruginosa. The biological characteristics of a algU mutant of X. fastidiosa were analyzed in the xylem sap, which includes nutrients within the host’s xylem-vascular system. The algU mutant had reduced abilities to adhere to a glass surface and form biofilm compared with the parent. Additional, the colony of algU mutant reduced the characteristic of twitching motility. These results suggest that AlgU of X. fastidiosa might regulate many virulence factors, which might enhance the adaptation of X. fastidiosa to xylem-vascular environmental stresses and causing disease symptom in plants.

The effect of sodium hypochlorite on the control of bakanae disease of rice caused by Gibberella fujikuroi

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Sodium hypochlorite has been used as a disinfecting and sterilizing agent against a broad range of seedborne pathogens. In order to develop effective control method for rice bakanae disease caused by Gibberella fujikuroi, the effects of sodium hypochlorite on antifungal activity was investigated in vitro and greenhouse conditions. The spore suspended in 0.1% sodium hypochlorite for 30 minutes did not germinated on the medium. Treatment of household bleach (about 5% sodium hypochlorite) for four hours was more effective to eliminate fungus from rice seeds infected with bakanae disease effective with treatment for two hour or seed treatment with Prochloraz, a common rice seed disinfectant in Korea. The elimination effect in the treatment of household bleach followed Prochloraz was significantly higher on the diseased rice seeds than Prochloraz treatment or household bleach only. When the diseased rice seeds were soaked into the twenty fold solution of household bleach for twelve hours, the disease incidence of rice seed was remarkably reduced up to 4.7% compare to 79.3% of non-treatment control. And the emergence rate of seed was higher at household bleach treatment compared to non-treatment control.

VCG and AFLP analysis of Fusarium oxysporum, the causal agent of koa wilt in Hawaii

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Acacia koa (koa), a tree native to Hawaii, has important roles in Hawaii. However, a serious dieback caused by Fusarium oxysporum (FO) makes the establishment of koa plantations difficult. The objectives of this study were: 1) to distinguish pathogenic and non-pathogenic strains of FO isolated from koa, and 2) to develop a rapid, economical method to detect pathogenic strains of FO in the soil. Pathogenicity tests were conducted with 46 Fusarium isolates collected from dieback koa specimens by adding 10⁶ spores of a fungal isolate to each of 10 koa seedlings. After 3 months, koa mortality ranged from 0–85% with 18 isolates killing no seedlings. Vegetative Compatibility Group (VCG) tests grouped the isolates into 16 VCGs with 2 major groups, VCG1 and VCG2, containing 8 and 16 isolates, respectively. Of the 46 isolates, 14 killed 50% or more of the seedlings tested, and of these 14 isolates, 12 belonged to VCG2. In AFLP analyses, isolates from the same VCG cluster with one another. Thus, strains of FO from dying koa trees may be pathogenic or non-pathogenic. By pairing representative strains from the VCGs with FO isolates collected from soil, the presence of pathogenic field strains can be confirmed. AFLP analyses of a larger set of strains may lead to the identification of conserved AFLP fragments that can serve as the basis for a diagnostic test for this pathogen. Testing the soil prior to planting will greatly increase the likelihood of establishing healthy koa plantations.

A volatile substance from Talaromyces sp. promotes the plant growth and blocks the disease development on several plants

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Talaromyces sp. a plant growth-promoting fungus was isolated from an agricultural field at Okayama, Japan. An isolate FS2 was identified as T. wortmannii based on ITS1 sequence and morphology. FS2 enhanced seed germination, root elongation and leaf growth of Brassica. The growth was also accelerated by volatile substance(s) emitted by FS2. GC-MS analysis of
the volatiles indicated that FS2 emitted at least seven terpenoids including β-caryophyllene that promotes the growth of Brassica, Crucifer and Nicotiana. Thus a part of the plant growth promoting effect of FS2 seems to be attributed to this terpene. Interestingly, we also found that Brassicae, Crucifer and Nicotiana pretreated with β-caryophyllene become tolerant to diseases caused by Colletotrichum higginsianum, C. lagenarium and Botrytis cinerea, respectively. Pretreatment with β-caryophyllene for 24 h decreased lesion expansion and infection rate by C. higginsianum on Brassica leaves. Similar results were obtained with the combination of Arabidopsis thaliana and C. higginsianum. Additionally, the yield of cucumber fruits increased significantly by treatment with β-caryophyllene. Taken together with an analysis of transcriptional activation of defense-related genes by RT-PCR, the results indicate that β-caryophyllene may act not only as a plant-growth promoting substance but also as an inducer or a priming compound for ISR. Based on these findings, we discuss the availability of PGPF-products for crop cultivation.

Risk analysis of native and ornamental plants for root infection and inoculum production from roots by Phytophthora ramorum

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Due to concern about possible spread of P. ramorum in the east through dispersal of inoculum in streams, over 45 species of plants were inoculated with P. ramorum and screened for root colonization and production of inoculum from colonized roots. All plants were compared to V. tumus, which served as a positive control. Plant species were considered for further testing if they initaited (but not necessarily showed root colonization to be at least 10%) and if roots gave off at least 10% of the inoculum produced by V. tumus plants inoculated at the same time. The ecological and commercial importance of the plant was also taken into account, as well as range and habitat. 16 plants were chosen for further tests: Arctostaphylos uva-ursi, four species of Camellia, Cornus serralis, Ilex glabra, Kalmia latifolia, Loniceria dioica, Nyssa sylvatica, Persea borbonia, four species of Quercus, and Rhododendron ‘Cunningham’s White’. These more formal tests analyzed inoculum over time using mixed-model regression analysis. Root colonization and total inoculum produced over the course of the experiment (corrected for dry root weight) was analyzed by a General Linear Models analysis. In these tests, Rhododendron ‘Cunningham’s White’, Camellia sinensis, Quercus prinus and Persea borbonia showed relatively high levels of inoculum production and root colonization, although no plant ranked as high as V. tumus.

Suppression of bacterial panicle blight of rice by pretreatment with various chemical compounds

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Bacterial panicle blight (BPB), caused by Burkholderia glumae and B. gladioli, is a serious disease of rice, which causes sterility and, consequently, failure of grain-filling. In spite of its economic importance, there are few effective control measures for this disease. Because numerous chemical compounds, including salicylic acid (SA), jasmonic acid (JA) and 2,6-dichlorobenzamide (2,6-DCB), are known to induce physiological immunities and acquired disease resistance, in many plants, we tested various chemical compounds for their ability to induce rice resistance to BPB in an attempt to develop a new control method for this disease. At 30% heading stage, panicles of the disease susceptible variety Trenasse were sprayed with SA, JA, ethephon, ascorbic acid (AA) and INAA 24 h prior to inoculation with B. glumae. BPB symptoms were rated using a standard rating scale, 0 – 9, at 10 days after inoculation. Among the tested chemical compounds, pretreatment of AA resulted in significant suppression of disease development and minimal yield reduction. These results suggest that AA could be useful for management of BPB in rice production. Currently, the mechanism of this protective effect of AA against BPB is being investigated and additional materials are also being tested.

Microarray reveals the role of auxin in mediating the interactions between Macrophomina phaseolina and Medicago truncatula

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Macrophomina phaseolina is a soil born necrotrophic fungus that causes charcoal rot disease in a wild range of plant hosts. Unlike most of fungal pathogens, M. phaseolina prefers hot and dry conditions. Current management approaches are very limited to effectively control this pathogen. The possibility of genetic engineering of disease resistant crop is hindered by our limited knowledge on charcoal rot disease. To better understand the molecular interactions between M. phaseolina with its plant hosts, we established a model pathosystem using Medicago truncatula. Using Affymetrix Medicago Gene Chip, we conducted a gene expression profiling experiment using RNAs isolated from plants that are infected with M. phaseolina at three different time points, namely, 24, 36 hour and 48 hour. The array data revealed up-regulation of genes in jasmonic acid and ethylene biosynthesis and signaling pathways. These results matched what we have found in our previously study. Interestingly, we also identified genes in auxin transport and signaling pathways that are regulated during the disease development. The expression of these genes is verified by quantitative real-time PCR. This finding has pointed to a new direction to better understand the compatible interaction between M. phaseolina and its plant hosts.

Mutation range leading to resistance to SDHI fungicides

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SDHI fungicides have a strong binding affinity towards a broad range of fungal succinate dehydrogenase enzymes, which inhibit electron transfer from succinate to ubiquinone and result in the inhibition of respiration. The current study focused on the resistance occurrence in Alternaria alternata on nut trees in California. More than 330 monosomic isolates from different treatments (before fungicide treatment, untreated, SDHI treated, SDHI plus QoI treated and anilinopyrimidines (AP) plus triazoles (DMI) treated) have been tested for the sensitivity to different SDHI fungicides (Boscalid, chlorothalonil) and 2,6-DCl (DMI) and 26 full-length begomovirus genome sequences. These results indicate strongly that SDHI+QoI select also for the highest diversity. Some mutations confers cross resistance among all the SDHI tested, other mutations have a differential response. These findings suggest that SDHI selection increases the diversity of mutations in fungal populations and subsequent selection increases the frequency of the most adapted (resistant) mutants in the treated pathogen populations.

Species diversity, phylogeny and genetic structure of begomovirus populations infecting leguminous weeds in Northeastern Brazil


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Begomoviruses are whitefly-transmitted plant viruses with a circular, ssDNA genome. Begomovirus diseases are a serious constraint to crop yields in most tropical and subtropical regions of the world. In Brazil, begomoviruses affect mostly common bean and tomato production. Weeds are considered to be begomovirus reservoirs as well as primary inoculum sources for epidemics in crops. We have carried out a survey of leguminous weeds (family Fabaceae) in four states of the Brazilian Northeast. A total of 59 samples were collected, and 26 full-length begomovirus genomes were RCA-amplified, cloned and sequenced. Sequence analysis indicated the presence of six distinct viruses, including four novel species. Macrosiphum lathoryzae was revealed as a common host for several of these viruses, and could act as a mixing vessel from which recombinant viruses could emerge. Phylogenetic analysis indicated that five of the viruses cluster with other Brazilian begomoviruses, likely of origin (Euphorbia yellow mosaic virus, EaYMV) clusters with viruses from Central and North America. Strong evidence of recombination was found among isolates of Macrosiphum lathoryzae var. MaYSV. The genetic structure of the MaYSV population indicates a high degree of genetic variability. Our results indicate that leguminous weeds are reservoirs of several begomoviruses, and could play a significant role in begomovirus epidemics both as inoculum sources and as sources of emerging novel viruses.

Diversity of plant pathogenic fungi associated with native Amazon forest species


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Phytopathology 101:S166

There is growing demand for native plant species for reforesting in Amazon region and as a result the number of nurseries have increased. The incidence
of leaf diseases are the major limiting factors for the successful raising of nurseries. The main objective of this investigation was to identify associated microorganisms and fungal pathogens causing leaf spots on native forest species of Amazon. The plants showing symptoms were collected during 2007–2009 in the nurseries of Urucu-Coari–AM/Petrolárias. The samples were kept in humid chamber and later isolations of fungi were made by direct and indirect methods. Sixteen fungi associated with disease symptoms were identified. Of these, 10 fungal pathogens were identified based on the pathogenicity tests. All fungi including pathogenic ones belong to group mitosporic, 44% being Hyphymycetes and 56% Coelomycetes. The most frequent genera were Pestalotiopsis (21.4%), Colletotrichum (17.9%), Beltramia (10.7%), Curvularia (7%), Heteroccephalum (3.6%), Phomopsis (3.6%), Stachybotrys (3.6%), Bipolaris (3.6%), Lasiodiplodia (3.6%), Cytospora (3.6%), Phyllosticta (3.6%), Meliola (3.6%), Myrothecium (3.6%) and Woldyromyces (3.6%). Colletotrichum sp. and Lasiodiplodia theobromae were pathogenic to Bellucia grossularioides. In Euterpe precatoria the lesions were caused by Colletotrichum sp. and Pestalotiopsis sp. The leaf spots in Aniba rosaeodora were caused by Miroteuchium.

**Inhibition of Magnaporthe oryzae and Rhizoctonia solani by Sarocladium oryzae, the causal agent of sheath rot in rice**

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Sheath rot of rice (Sarocladium oryzae) has been increasing in significant proportions in different rice growing States of Brazil. The antagonism of 16 isolates of S. oryzae obtained from infected grain and sheath was studied utilizing Magnaporthe oryzae as test organism. A clear inhibition zone was formed between the two rice pathogens in a dual culture test in Petri plates containing PDA. The isolates showed significant differences in relation to the inhibition of M. oryzae. Similar results were obtained in tests conducted with culture filtrates. The pathogenicity of isolates was tested on rice cultivar Metica-1 in the green house. The isolates were multiplied in autoclaved sorghum grains and 69 days old plants were inoculated by inserting a single grain infested with mycelium and spores between the top most leaf sheath and culm. The disease was assessed seven days after inoculation utilizing lesion length as a criterion for determining the aggressiveness of the test isolates. The isolates exhibited significant differences in lesion length on leaf sheaths of inoculated tillers. There was, however, no correlation between the degree of inhibition of M. oryzae in vitro and aggressiveness of S. oryzae isolates in inducing sheath rot symptoms. Several isolates of S. oryzae also showed inhibition of mycelium and sclerotia of Rhizoctonia solani on PDA in dual culture method as well as by using culture filtrate in repeated laboratory assays.

**Vermicompost tea for control of Phytophthora nicotianae in pineapple**

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Heart rot of pineapple, caused by Phytophthora nicotianae, can be devastating to low-acid pineapple hybrids which are more susceptible than the Smooth Cayenne. In field tests, hybrid grown in a vermicompost amended soil was more vigorous than those grown in soil with no amendment. Five bands were shown to have a strong association with the long lesion length phenotype. This should make it possible to identify individual genes that influence virulence to pine in G. circinata.

**Effect of bed height and soil amendments on survival of southern highbush blueberry cultivars in Phytophthora spp. infested soils in Mississippi**

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Phytophthora root rot is an important disease of blueberry and is most severe when plants are grown in wet soils with poor drainage. Symptoms include small, yellow or red leaves, lack of new growth, root necrosis, and a smaller root system than that of healthy plants. Four studies were conducted in south Mississippi to evaluate the effect of bed height (flat or raised bed) and soil amendments (none, peat moss, or pine bark) on the survival of 19 southern highbush blueberry cultivars transplanted into soils infested with the root rot pathogen, Phytophthora cinnamoni. Plants were rated twice a year for overall vigor. The most vigorous cultivars were: Southmoon (2005 study), Gulfcoast (2006 study), and Springhill (2008 study). In the 2005 and 2006 studies, plants grown on raised beds were more vigorous than those grown on flat beds due to root rot pressure. On the whole, plants grown in a vermicompost amended soil was more vigorous than those grown in soil with no amendment. In the 2008 study plants grown in pine bark amended soil were more vigorous than those grown in peat moss amended soil. However, in each study plant vigor declined each year, and most plants died within three years whether they were planted on raised or flat beds and whether they received any soil amendments or not. No cultivar thrived in any study. These studies demonstrate that southern highbush blueberries should not be planted in soils known to be infested with P. cinnamoni.

**Molecular and biochemical characterization of resistance to Botrytis cinerea among the Solanaceae**

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Grey mold caused by the necrotrophic fungal pathogen, Botrytis cinerea, is a major economic concern for tomato (Solanum lycopersicum, S.) production. Grey mold can result in lesions on stems, leaves, and flowers of infected tomato plants as well as fruit rot both pre and post-harvest. Although all known cultivars of tomato are susceptible to grey mold, Solanum lycopersicoides (Slo), a wild relative of tomato, is extremely resistant. The objectives of this study are to characterize the molecular and biochemical basis of resistance of Slo to grey mold as well as to gain new insight into host responses to necrotrophic pathogens. To this end, next generation sequencing
(454) was used to generate transcriptomics data from Slo prior to and 24 and 48 hours after inoculation with \textit{B. cinerea}. Bioinformatics analysis of the transcriptomics data revealed numerous genes in \textit{Slo} that are upregulated in response to infection by \textit{B. cinerea}. Additionally, unbiased metabolic profiling techniques utilizing mass spectrometry were developed to identify defense-related compounds differentially expressed in \textit{Slo} before and during infection. This research will help to uncover the molecular and biochemical mechanisms underlying resistance to necrotrophic pathogens.

**Screening of a Valencia peanut core collection for resistance to \textit{Sclerotinia sclerotiorum}**

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Phytopathology 101:S168

**Development of molecular diagnostic markers for \textit{Xanthomonas translucens}**

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Phytopathology 101:S168

\textit{Xanthomonas translucens} (Xt) is the causal of bacterial streak and black chalk in small grains. The bacterium is seed borne and there is need for reliable diagnostic methods to detect and differentiate pathogens that may contaminate seed lots. Also, the use of pathovar designations within \textit{Xt} have been inconsistent and further characterization of this species and its pathogens is warranted. We developed draft genome sequences of 12 \textit{Xt} isolates representing four different pathovars using Illumina sequencing technology. Genomes were assembled with the short read sequence assembler Velvet. The gene finder GLIMMER and MAKER genome annotation pipelines were used for gene prediction and annotation in each genome. Isolates of \textit{Xt} pv. \textit{translucens} and \textit{undulosa} were distinct from isolates representing pv. \textit{poeae} and pv. \textit{translucens} based on unique sequence typing. Eight PCR primer sets were developed from unique regions in the genome. These primers amplified geographically diverse strains of \textit{Xt} pv. \textit{translucens} and \textit{undulosa} collected from wheat and barley, but not other bacterial plant pathogens including \textit{Xanthomonas}, \textit{Pseudomonas}, \textit{Burkholderia} and \textit{Erwinia} spp. Several of the primer sets have been successfully incorporated into a multiplex PCR design to expedite identification of \textit{Xt}.

**Chromobacterium sensu lato isolated from native and commercial cranberry with potential for biological control of \textit{Phytophthora} root rot**

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Phytopathology 101:S168

Disease and pest suppression with natural strains of bacteria has the potential for decreasing agricultural reliance on pesticide inputs. A primary screen of cranberry (\textit{Vaccinium macrocarpon}) rhizosphere bacteria from both native and commercial cranberry beds in eastern Massachusetts yielded a new type of \textit{Chromobacterium} (\textit{Neisseriaceae}, \textit{β-proteobacteria}) which produces the dark-purple pigments violacein and deoxyviolacein. \textit{Chromobacterium sp.} was tentatively identified by pigment production, 16S ribosomal gene sequence analysis, and phenotypic characteristics. How PCR primer sets were developed from unique regions in the genome. These primers amplified geographically diverse strains of \textit{Xt} pv. \textit{translucens} and \textit{undulosa} collected from wheat and barley, but not other bacterial plant pathogens including \textit{Xanthomonas}, \textit{Pseudomonas}, \textit{Burkholderia} and \textit{Erwinia} spp. Several of the primer sets have been successfully incorporated into a multiplex PCR design to expedite identification of \textit{Xt}.

**Kermes scale (\textit{Allokeymes} sp.) and the dry leaf pathogen \textit{(Brenneria quercina)} associated with a decline of red oak species in Colorado**

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Northern red oak (\textit{Quercus rubra}) and pin oak (\textit{Q. palustris}), while not native to Colorado, are a small, but important component of the urban tree landscape. The kermes scale (\textit{Allokeymes} sp.) is a common insect pest associated with these tree species. Scale feeding results in reduced tree vigor, twig dieback and witch’s broom growth on some oak species. Historically, tree damage associated with kermes scale infestations has been attributed exclusively to insect feeding. However, we have noted the presence of small cankers and extensive gummosis at many of the scale feeding sites for several years. Gummosis is so copious in some years that it drips from branches onto sidewalks and parked cars creating a nuisance. In 2010 the bacterium \textit{Brenneria quercina} was consistently isolated from canker margins and bacterial ooze. Identification of the bacterium was confirmed by sequence analysis of a 1.5KB region of 16s rDNA. This bacterium was first described in California in 1967 as causing a disease of acorns of two native California oaks. The disease was named dryly nuts because of the copious bacterial ooze that leaked from infected acorns. This bacterium has not previously been associated with kermes scale feeding or dieback of northern red and pin oaks, nor has it been reported outside of California or Oregon in the United States, although it is associated with an oak decline in Europe.
Pseudomonas did not have an effect on plant growth, others (like AWR5) dramatically reduced the pathogen multiplication. When AWR proteins were transiently expressed in non-host Nicotiana spp., necroses took place to different extents. AWR5 induced the strongest necrosis, resembling an HR phenotype which was confirmed by TB/DAB staining and by RT-PCR of specific HR marker genes. In summary, AWR effectors play an important role in pathogen resistance and might also be recognised as they reduce P. syringae virulence and trigger an HR-like phenotype in non-host plants. Deciphering effector function will open promising avenues towards the design of new strategies to control R. solanacearum.

Formation of chlamydospore-like structure in the ascomycete fungus Gibberella zeae

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The homothallic ascomycete fungus Gibberella zeae is an important pathogen on major cereal crops. In this study, we found that conidia of G. zeae were readily changed to chlamydospore-like structures in minimal conversion medium supplemented with mannitol. Chlamydospores are enlarged, wall-walled vegetative cells with various forms and are produced from many fungal species. These structures accumulated high level of glycogen, lipid, and chitin which might be functional for stress resistances including UV, heat, and drought. We also found that various biological processes, including signal transduction, acetal-CoA production, and chitin synthesis, are involved in chlamydospore-like structure formation. Based on these characteristics of chlamydospore-like structures, these might be produced in field condition at mild temperature and could be used for survival in hot and drought field conditions.

The occurrence and management of brown planthopper, Nilaparvata lugens (Stål), in Korea

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Phytopathology 101:S169

The brown planthopper (BPH), Nilaparvata lugens, is a migrant pest from tropical into subtropical and temperate areas every year and presents a great threat to rice production in Asia. A computer model was developed to simulate the migration waves and the impacts on the temperate rice ecosystem in Korea. The simulations showed that 1) each migration wave had different mass distribution and immigration areas; 2) the vertical air current value in the planthoppers’ take-off and landing area was only several centimeters per second; 3) the main migrant source into southern Korea was from the eastern part of Guangdong Province and south-eastern part of Fujian Province in China. The population dynamics of the BPH after migration were closely executed in both sprayed and unsprayed rice fields. Early immigration contributes more to the population build-up and damage in late season than the amount of migration. Severe BPH resurgence occurred in the sprayed rice fields in both years of 2005 and 2006. The peak density was 4 to 20 times higher in the sprayed field than in the unsprayed field. The effect of root zone application of some systemic insecticides was tested as one of the BPH management strategies. Dicofol and furfuran root zone treatment was the most effective in increasing BPH mortality and reducing the percentage of eggs hatched. The treatment had not been effective on spiders while the broadcasting and foliar spray killed all ambush and hunting spider groups within one day after application.

Eradicating grapevine disease with minimal economic impact

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Phytopathology 101:S169

Eradicating exotic grapevine diseases using current strategies, which include complete removal of affected and suspected vines, can incur significant costs to growers and the industry. An alternative strategy has been developed through collaboration between Australia and the U.S.A. which minimises the process of eradicating a pathogen while minimising the economic cost of returning the crop to its previous quality and production levels. In Australia, black spot disease (grapevine anthracnose, Elsinoe ampelina) was used as a model to evaluate a drastic pruning eradication strategy developed for the fungal disease black rot (Guignardia bidwellii), exotic in Australia but endemic in eastern U.S.A. The protocol involved cutting off vines at the top of the trunk and removing low water shoots; removing debris from the ground beneath and between vines; mulching the vineyard floor; and applying a targeted fungicide program. The same protocol was evaluated in a black rot-infested vineyard in New York. Following two seasons of weather conditions conducive to black rot development, no disease was detected on treated vines, whereas leaf and fruit infections developed on the control vines. These results confirmed the efficacy of the protocol for eradicating black rot from vineyards. The protocol provides an alternative to razing a vineyard following an incursion of black rot in Australia. This general strategy may have potential for use on other diseases of grapes and other perennial crops.

Isolation of double-stranded RNA mycoviruses in Macrophomina phaseolina isolates in Iran

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Macrophomina phaseolina, the causal agent of charcoal root rot disease, is one of the soil borne plant pathogens in the tropical and subtropical regions. This pathogen has a wide host range. Biological control of some fungal pathogens by mycoviruses has been reported in the world. Thus, this study was conducted to explore the possibility of using mycoviruses as a new biocontrol agent of charcoal root rot disease. For this purpose, the presence of dsRNA was surveyed in 66 isolates of M. phaseolina collected from different regions of Iran. At first, the isolates were grown on potato dextrose broth (PDB) and stirred at 25°C for two weeks for maximized mycelial mass production. DsRNA fragments were extracted from the mycelial mass using STE buffer and CF-11 cellulose chromatography. DsRNA fragments ranging from 0.9 to 12 kb in size were detected in 18 out of 66 surveyed isolates. These isolates had been isolated from sugar beet, soybean and sesame hosts. The presence of dsRNA was confirmed by RNase-A at high and low SSC buffer concentration. This is the first report on the detection of viral dsRNAs from M. phaseolina in Iran.

The effect of dsRNA mycoviruses of Macrophomina phaseolina on pathogenicity, lactase activity, mycelial growth and microsclerotia production

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Various viral genomes from phytopathogenic fungi have been reported worldwide. The most common of these genomes are dsRNAs that in some fungi are associated with hypovirulence and have been used or proposed as biological control agents. In this study 66 Iranian isolates of Macrophomina phaseolina, the causal agent of charcoal root rot disease, were studied for dsRNA infection and investigated the effect of these viral genomes on some phenotypic characteristics. Extraction and purification of dsRNA was performed using CF-11 cellulose chromatography and confirmed by digestion with specific nuclease (RNase A). In 27.2% of these isolates dsRNA fragments ranging from 0.9 to 12 kb in size were detected. Partial curing of dsRNA fragments in 11 isolates out of 18 dsRNA containing isolates using cycloheximide (100 µg/ml) was successful. Eleven isolates having dsRNA and 11 dsRNA-free isolates were evaluated in more details. The results indicate that dsRNA can have different effects on morphology and biology of the fungus. In some isolates dsRNA increased the mycelial growth, lactase activity and pathogenicity and in the others dsRNA decreased the above characteristics. In a few of them, there was no correlation between the presence of dsRNA and mentioned characteristics. DsRNA had no effect on microsclerotia production. In 4 isolates dsRNA reduced virulence towards sugar beet. These results indicate an association between the presence of dsRNA and hypovirulence.

Efficacy of silk channel injections with insecticides for management of lepidopteran pests of sweet corn

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The primary pests of sweet corn in Georgia, U.S.A., are the corn earworm, Helicoverpa zea, and the fall armyworm, Spodoptera frugiperda. Control of these pests requires multiple insecticide applications from silking to harvest, with commercial growers frequently spraying daily for 16 to 18 days. Injection of oil into the silk channel 5 to 8 days after silking initiation has been used to suppress damage by these insects. Initial work with this technique in Georgia provided poor results. Subsequently, a series of experiments was conducted to evaluate the efficacy of silk channel injections...
as an application methodology for insects. A single application of spinosad or chlorantraniliprole, at reduced rates as compared to common foliar applications, provided excellent control of lepidopteran insects attacking the ear tip and suppressed damage by sap beetles. Oil and oil plus a *Bacillus thuringiensis* insecticide provided minor suppression. The use of water plus a surfactant as the carrier also showed promise and avoided potential adverse effects of oil on pollination. This methodology is labor intensive but requires a single application of insecticide at reduced rates applied approximately two weeks prior to harvest. This methodology is not likely to eliminate needs for foliar applications because of other insect pests but would greatly reduce the number of applications required and may prove particularly useful for small acreage growers.

**Refined empirical models for predicting *Fusarium head blight* epidemics in the United States**


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Fusarium head blight (FHB) of wheat causes significant yield and grain quality losses in U.S. wheat. A web-based FHB forecasting system (http://www.wheatscab.psu.edu/) was developed in 2006 to predict outbreaks early enough to apply preventative fungicides. Further enhancements to existing models and the development of new models are underway using an expanded dataset from 1982 to 2009 containing 527 observations over 15 states. Multiple imputation was used to fill missing values in the data matrix. Current model parameters have been updated using a bootstrap sample of the imputed datasets. This analysis suggests that a model currently used for spring wheat may also perform well for winter wheat. Subset selection algorithms identified several other weather-based predictors that may be of potential use for improving prediction models for FHB. A comparison of the models currently in use, updated models, and newly developed models will be presented.

**Diversity of vegetative compatibility groups in Michigan populations of the chestnut blight fungus, *Cryptonectria parastica*, 1996 to 2009**

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Hypoviruses change virulence of *Cryptonectria parastica* allowing infected chestnuts to recover. Hypovirus spread is limited by vegetative incompatibility in the pathogen, which is governed by a bi-allelic, six locus system. London and Alleles cause apoptosis where hypahe fuse, inhibiting the spread of hypoviruses. Vegetative compatibility (vc) diversity was characterized in seven Michigan *C. parastica* populations in both 1996 and 2009. Four populations are causing major epidemics and lack hypoviruses. The three remaining populations have hypoviruses and chestnuts are recovering. Thirty single-spore isolates from each population were scored for vegetative compatibility. Each recovering population contained vc groups that were unique to the site. Two recovering sites were dominated by a single vc group which was stable over time. A third site, Frankfort, was more variable; each recovering population contained vc groups that were variable both spatially and temporally because ascospores are likely to migrate among populations and may cause regular recombination.

**A new selective medium for isolation of *Rhizoctonia* spp. from soil**

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Several assay methods, including elutriation, toothpick, and soil pellet, were evaluated for isolation of *Rhizoctonia* spp. from rice, cotton, and soybean fields in Arkansas. Growth of *Trichoderma* spp. and Mucorales made isolation and quantification of *Rhizoctonia* spp. difficult in *Ko* and *Hora* medium. *Ko* and *Hora* (KH), ethanol potassium nitrate medium with 2% (EPN1) and 5% (EPN2) ethanol, and various chemicals in water agar were used to evaluate average daily growth of *Rhizoctonia* spp. and other fungi. *Trichoderma* spp. were the fastest growing isolates in KH, while growth did not differ between *Rhizoctonia solani* AG4 and an isolate of *Rhizomucor variabilis*. Average daily growth of all isolates in EPN1 and EPN2 were non-significant although one *Trichoderma* sp. was completely suppressed. A new selective medium, "TS", based on chemicals that improved inhibition of *Trichoderma* spp. and *R. variabilis* was developed consisting of 7 g/L Moorehead agar, 0.09 g/L metalaxyl (Apron 50WP), 0.229 ml a/L potassium phosphate (Alude), 100 µl of a rifampicin solution in dimethyl sulfoxide (10 mg/ml), 0.25 g/L ampicillin salt, and 0.4125 µl to 2.0625 µl a/L thiophanate-methyl (3336F). The level of thiophanate-methyl depended on the level of suppression needed for *Trichoderma* spp. and desired growth of *Rhizoctonia oryzae*. The "TS" medium has been effective in assaysing populations of *Rhizoctonia* spp. over fields and methods.

**Identification of potential virulence genes of *Candidatus Liberibacter asiaticus* differentially expressed in citrus and psyllids, using real-time PCR**

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Citrus greening is a destructive disease of citrus in the United States, and is caused by the bacterium, *Candidatus Liberibacter asiaticus*. This disease is transmitted by the Asian Citrus Psyllid, *Diaphorina citri*. The pathogen causes severe symptoms in plant compared to the psyllid. We hypothesized that a number of pathogenicity/virulence related genes of the bacterium would be overexpressed in planta, compared to the psyllid. To test this hypothesis, quantitative real-time PCR assays using total RNA isolated from infected plants and psyllids were conducted. Gene specific primers were used to check the expression of 560 genes in *Ca*. *L. asiaticus*. The genes showing a differential expression of two fold or more in either the plant or psyllid were categorized into Clusters of Orthologous Groups of protein functional categories. Potential virulence related genes including hypothetical genes, which were overexpressed in *planta*, were selected. Differential expression of these selected genes were also evaluated in susceptible and tolerant varieties of *Ca*. *L. asiaticus* infected citrus. Potential virulence related genes were then screened on *Nicotiana benthamiana* plants for symptom expression, using transient assays. The results from this study will be useful in identifying the potential virulence genes involved in symptom expression and survival of this pathogen in planta.

**Assessing the genetic basis of resistance to rice sheath blight**

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Sheath blight (ShB) caused by *Rhizoctonia solani* is one of the most important diseases of rice. Resistant rice varieties would represent the best possible disease control option, both environmentally and economically. To date, no immune rice accession has been reported, but some cultivars with partial resistance to ShB have been identified. Development of quantitative resistance to ShB is a complex quantitative trait, determined by polygenes/QTLs. Plant height, heading date, and other canopy traits play an important role in field resistance to ShB. QTL for ShB resistance have been identified and mapped, some of them being co-localized with QTL governing morphological traits and heading date. Whether or not such colocalizations are due to pleiotropy or tight linkage remains a question. Here we report a study aiming at identifying genes for quantitative resistance, and at further analyzing the genetic basis of resistance to the disease. We phenotyped 163 rice accessions of *Oryza sativa L.*, many of which have been reported to be partially resistant to ShB. Accessions were screened using two complementary methods, in microfield tests and with a detached tiller assay. Morphological traits were also assessed. The accessions were further genotyped with SSRs reported to be linked with ShB resistance and candidate genes associated with resistance. The results of this study are discussed with respect to the genetic basis of resistance to ShB, and implications on breeding for ShB resistance.

**An in vitro evaluation of chemical and biological agents for control of Botryosphaeria species**

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A major limiting factor in the successful culture of fruits such as pome fruit (apple, pear), stone fruit (peach, nectarine, plum), blueberries and persimmon in Florida are the diseases incited by the fungal genus Botryosphaeria. Botryosphaeria have a host range encompassing at least 100 plant species, and for most plant species there is currently no adequate chemical control. The fungicides Azoxyostrobin, Chlorothalonil, Copper Hydroxide, Flutriafol, Propiconazole, Pyraclostrobin, Tebuconazole, Tetraconazole and Thio-
phenate-methyl were tested for efficacy against B. rhodina, B. obtusa, B. dothidea and Neofusicoccum ribis in vitro. The fungicides tested generally offered only partial reduction in fungal growth even at relatively high (10 to 25 mM) concentrations. Naturally occurring phenolic compounds from plants were also tested, including vanillic acid, syringic acid, catechol, vetaric acid, 2,6-dimethoxy benzoic acid, ferulic acid, benzoic acid, 2,6-dimethoxy phenol, p-coumaric acid and guaiacol. The efficacy of the phenolic compounds varied with the specific compound and with Botryosphaeria spp. Inhibition of mycelial growth was dose-depandant ranging from 48–100% inhibition with benzoic acid and 69–88% with guaiacol in different Botryosphaeria spp. Biocontrol agents (Bacillus spp.) were also tested in vitro and exhibited potential activity in limiting the establishment of the Botryosphaeria spp. in vitro.

Discovery of key pathogenesis-associated genes among predicted transcription factors in the plant pathogenic fungus, Alternaria brassicicola

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The necrotrophic fungus Alternaria brassicicola causes black spot disease of Brassicaceae family. Several physiological and morphological characteristics have been hypothesized to be involved in fungal pathogenesis, such as production of cell wall-degrading enzymes, proteases, secondary metabolites, and a fast growth rate. These characteristics are believed to be coordinately regulated by transcription factors and little is known about their regulatory mechanism. In this study we created knockout mutants of the genes encoding for 173 of 380 predicted transcription factors of A. brassicicola. Our bioassays on green cabbage leaves identified 11 genes strongly associated with pathogenesis. One of the genes caused a loss of pathogenicity, another caused 100% increase in virulence, and the others caused a reduction in virulence of up to 90% compared to the wild type, as measured by lesion size. Ten of these eleven genes were novel virulence-factors. Only one of the genes has been previously identified as a virulence factor in other fungi. The functional genomics approach by systematic targeted gene knockout proved to be useful in discovering transcription factors associated with pathogenesis. Detailed characterization of the functions of these transcription factors will shed light on the regulatory mechanisms of pathogenesis and allow the design of efficient strategies specific to the management of necrotrophic fungi.

Role of rhizosphere microbial communities and nematodes in SDS development and/or suppressiveness in soybean cultivated fields

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Bioret and abiotic characteristics of the soil were being evaluated in relation to the incidence and severity of Sudden Death Syndrome (SDS), caused by Fusarium virguliforme, in soybean fields. In order to compare soil biota across a large number of samples and to overcome the difficulty of culture dependent techniques, we have used a metagenomic approach employing PCR-DGGE targeting 16s rDNA, 18s rDNA and internal transcribed regions (ITS) in total rhizosphere DNA. Soil was collected from fields with high SDS incidence (approximately 75%). The samples were collected from areas of high SDS incidence (hot-spots) and areas of low SDS incidence in the same fields. Fingerprint analysis of Fungi-DGGE showed a distinguishable pattern of banding separating samples from hotspots from those originating from non-hotspots in the same field. Quantitative PCR was used to assess the extent of F. virguliforme presence in tested soil samples. Samples from hotspot areas had significantly higher levels of F. virguliforme than those from low SDS incidence sites in the same field. This suggests that DGGE banding patterns may be used as indicators of SDS incidence and aggressiveness in soybean fields. DGGE followed by Illumina sequencing of small ribosomal DNA (16S, 18S and ITS) will enable us to establish a bank of information about microorganisms communities structures and functions in soil, their interaction with F. virguliforme and their possible effect on the incidence of SDS.

Homalodisca vitripennis reovirus polymorphism validates timing and limited introduction of glassy-winged sharpshooter to California

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Homalodisca vitripennis reovirus (HoVRV) is a phytoparovirus species infecting the glassy-winged sharpshooter (GWSS), an invasive insect introduced to California. Complete genome sequences of five Californian and four southeastern U.S. isolates of HoVRV were evaluated for polymorphism. Nucleotide sequence diversity was 10-fold less for HoVRV in California compared to HoVRV from the southeastern U. S. Phylogenetic analysis of each dsRNA segment indicated that the Californian isolates grouped as a monophyletic lineage. To sample diversity at single locations, dsRNA segment 11 was sequenced for nine additional isolates each from Riverside, CA and Johnston Co., NC. Whereas 9 of 10 Riverside isolates were identical (the tenth varied at one position), Johnston Co. isolates varied by up to 1.5%. Coalescent analyses estimated median population age at 11.6 to 26.3 years, with the most appropriate model (exponential growth) yielding a median age of 19.9 years. Estimates of median molecular clock rate for the Californian population translated to 0.4 to 1.4 substitutions/gene/year. Collectively, the results indicate that HoVRV diversity in the native range (southeastern U. S.) was high relative to a newly established population (California), and that the Californian population of HoVRV was subjected to a bottleneck coinciding with introduction of GWSS circa 1988.

Evolutionary history and species boundaries of the citrus brown spot fungus

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Traditional species concepts are difficult to apply to closely related, asexual taxa because of the lack of an active sexual phase and a paucity of morphological characters. Phylogenetic species concepts such as genealogical concordance phylogenetic species recognition (GCP SR) have been extensively used; however, methods that are able to incorporate uncertainty of species boundary estimation may more accurately and objectively delineate species groups. Using a worldwide sample of the citrus brown spot fungus (Alternaria alternata sensu lato), the evolutionary histories of an endo-polymagulatoriae gene, two SCAR loci, and two microsatellite flanking regions were estimated using the coalescent. Species boundaries were compared using four methods: concatenation, GCP SR, and two methods that incorporate gene tree uncertainty, the “minimize deep coalescence” (MDC) and the Bayesian Estimation of Species Trees (BEST) methods. Coalescent analyses showed patterns of divergence influenced by incomplete lineage sorting and recombination among four phylogenetic lineages. Divergence of the citrus 2 lineage from the other lineages was well-supported with divergence times estimated between 1-12 my before present, predating the migration of citrus from SE Asia. Two species were identified using concatenation and GCP SR, but only a single species was detected using BEST and MDC.

Virulence of Fusarium root-disease pathogens (Fusarium oxysporum and F. commune) to Douglas-fir (Pseudotsuga menziesii)

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Fusarium species can cause damping-off and root rot of young conifer seedlings, resulting in severe crop and economic losses in forest nurseries. Management of Fusarium disease in forest nurseries could be greatly enhanced by accurate identification of the Fusarium species, especially highly virulent isolates of F. commune. The primary objective of this study was to test the roles of F. commune and F. oxysporum in disease of Douglas-fir (Pseudotsuga menziesii) using unknown Fusarium isolates under in vitro and greenhouse conditions. Fusarium isolates were collected from healthy and diseased seedlings of Douglas-fir and western white pine (Pinus monticola) from nurseries in Idaho, U.S.A. In vitro and greenhouse virulence tests were completed on Douglas-fir germinated seedlings. The virulence tests demonstrated that F. commune is a highly virulent pathogen, whereas F. oxysporum is mildly virulent to Douglas-fir germinated seedlings. In addition, a species-specific diagnostic primer set was developed to detect and identify isolates of F. commune. With this information, nursery managers could more effectively deploy an appropriate disease-management strategy. This is the first report of direct evidence that F. commune can cause damping-off disease on Douglas-fir seedlings under greenhouse conditions.

Use of massively parallel sequencing as a diagnostic tool

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Current methods for the detection of unwanted pathogens in plants include hybridization probes. Strict physical requirements limit the number of probes used in a single test. To alleviate this limit, a new detection method is being developed, using massively parallel sequencing (MPS) for detection. In this method, pathogen-specific sequences are used in BLAST searches of a database of reads attained from a MPS run. Without physical limits, dozens of queries are possible for virus pathogens, and thousands for bacteria. Mock sample databases were generated to find optimal parameters for the searches. The databases were generated using a MPS simulation program, and contained both host and pathogen sequences. The proportion of pathogen reads in the databases varied from 25% to <0.5%. Queries were designed with various lengths (20 – 140 nt). Three criteria were considered to find an optimal length of given length, the number of hits per query, and the number of queries that received one or more hits. An optimal length of 60-80 nt was determined. A cut-off E-value of 1e-3 for calling hits was determined by looking at the true/false hit ratio. The specificity of this method was also tested by querying databases that include near neighbors to the target pathogens. The number of matches from these searches was similar to the number of matches found when querying a pathogen free database, as opposed to the target pathogen database.

**Factors influencing efficacy of plastic shelters for control of bacterial blight of lilac**

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Phytopathology 101:S172

Plastic shelters are thought to reduce bacterial blight by protecting plants from freezing events were frequent (>20/seas on), but average low air temperatures. **Bacterial blight of lilac**

Epileptic R. solani and Pythium ultimum in soybean or cotton

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Phytopathology 101:S172

Regalia®, an extract of giant knotweed Reynoutria sachalinensis, induces resistance in plants by increasing production of phytoalexins and other defensive compounds that are toxic to red spider mite (or other) pathogens. In this study, Regalia® was applied as seed coating or drenched in soil to control soilborne disease caused by Rhyzoctonia solani or Pythium ultimum in soybean and cotton. Regalia® alone, or mixed with azoxystrobin (Quadris®), fludioxonil (Scotol®), or mfenoxan (RidomilGold®) were coated with Sepritet® 1171-O on soybean or cotton seeds. The treated seeds were seeded in soil infested with R. solani or P. ultimum. Results show that soybean or cotton seeds coated with Regalia® had greater or significantly greater emergence than that of the untreated control. Regalia® showed synergy when mixed with the synthetic fungicides. Drenching with Regalia® also significantly increased emergence and growth of soybean planted in soil infested with R. solani.

**Diversity and distribution of Iris yellow spot virus (genus Tospovirus) infecting onion in Eastern Africa**

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Iris yellow spot virus (IYSV; family Bunyaviridae, genus Tospovirus), transmitted by thrips (Thrips tabaci) is an important viral pathogen of onion in many parts of the world. In Africa, IYSV was reported from South Africa, and Reunion Island. Our recent surveys found that IYSV is present in most onion-growing regions in Kenya and Uganda and its incidence ranged between 27.38 – 72.03%. IYSV was confirmed with specific ELISA tests and RT-PCR with IYSV specific primers followed by cloning and sequencing of the amplicons. The IYSV-Kenya isolate (HQ711616) had the highest nucleotide sequence identity (97%) with the corresponding region of IYSV-Sri Lanka isolate (GenBank # GU901211) and the IYSV-Indian isolates (EU310287 and EU310290). The IYSV-Uganda isolate (HQ711615) showed the highest nucleotide sequence identity (95%) with IYSV-Sri Lanka (GenBank # GU901211) and IYSV-India isolates (95% with EU310274 and EU310297). The maximum entropy method (Maxent2) was used to model the IYSV geographic distribution based on the Bioclimatic data layer from the WorldClim (www.worldclim.org) version 1.4 dataset and the presence of IYSV in Eastern Africa, South Africa and Reunion Island. Results from the Maxent2 showed that most of the regions in Eastern Africa and Ethiopia are conducive for IYSV occurrence and outbreaks.

A quantitative PCR assay for the detection of phytoplasmas causing almond brownline, peach yellow leafroll, and pear decline diseases in California

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Phytopathology 101:S172

Phytoplasmas cause several important diseases in California’s almond and fruit tree orchards. A phytoplasma associated with almond brownline (ABL) disease, peach yellow leafroll phytoplasma (PYLR-P) and pear decline phytoplasma (PD-P) are genetically related based on homology in the 16S-23S rDNA spacer region. In an attempt to detect these three phytoplasmas, a
primer pair was designed to amplify a 530-bp product from the 16S-23S rDNA spacer region. Using this primer pair and SYBR Green, a quantitative PCR (qPCR) assay was developed to facilitate detection and analysis from multiple samples. A detection limit of 100 copies was achieved in assays using nucleic acid extracts from almond leaves spiked with cloned target DNA from PyrL-P. The qPCR assay detected PyrL-P in almond and peach trees, PD-phytoplasma in pear trees, but not a genetically different X-disease phytoplasma in cherry trees. This new assay was also able to detect PYLR-phytoplasma in dormant peach buds from infected trees and is expected to aid in the detection and amplification of ABL-P, PYLR-P and PD-P in almond, peach and pear trees, respectively.

Genetical, biological and pathological characters of Japanese potato strains of Ralstonia solanacearum

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Strains of potato bacterial wilt pathogen Ralstonia solanacearum (Rs) in Japan, were surveyed about biovar, phylotype, and DNA fingerprints (rep-PCR), and/or endoglucanase gene (egl) sequence. Rs were isolated from potato fields in southwestern, warm, temperate regions. Of the 188 isolates, 74 were analyzed by rep-PCR), and/or endoglucanase gene (egl) sequence. Rs were isolated from inoculation as seedlings with different cultivars/strains evaluated in field as matured plants and artificial soil conditions in pots. Only half of them showed similar response in field and pots for the pathogen. Among 19 forage corn strains, only half of them showed similar response in field and pots for the pathogen. Differences in growth, disease severity varied and depended on the Rs strains as well as the plants tested. Strains of phylotype IV, biotype n2 strains. None of the strains belonged to phylotype I, biovar 3 or 4 wilted in pots, only half of them showed similar response in field and pots for the pathogen.

Pythium root rot of corn in Japan; unique symptom climb up the mature stem

K. SUGAWARA (1), T. Tsukiboshi (1), T. Kikawada (1), H. Tamaki (1), S. Mitsuhashi (1), S. Morita (1), I. Okabe (1)  
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Detection of latent infection of wheat leaves caused by Puccinia striiformis f. sp. tritici using single-tube nested PCR

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PEOPLES REP OF CHINA

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Wheat stripe rust, caused by Puccinia striiformis f. sp. tritici (Pst), is one of the most important diseases of wheat worldwide. Early detection of latent infection of wheat seedling is critical to estimate initial inoculums potential of epidemics and effective control. In order to improve the sensitivity and facilitate the procedure of common nested PCR Two pairs of Pst species-specific primers including external and internal primer pairs with different annealing temperatures were designed according to the beta-tubulin gene sequence of Pst. The annealing temperature of external primer pairs was 12°C higher than the internal ones. Sensitivity test demonstrated that the single-tube nested PCR could detect as low as 20 fg template DNA and about 100 times more sensitive than the standard PCR. And latent infections from wheat seedlings could be detected as early as 24 h after inoculation. This study provides a rapid and simple method to detect and estimate latent infections level of seedlings in the field.

Fungal communities on strawberry roots and in soils amended with mustard meal (MM)

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Phytopathology 101:S173

Community analysis of soil and root fungal pathogens is critical for understanding pathogen ecology and the impact of MM amendments on pathogen communities. The dynamics of microbial communities was monitored in soils amended with MM or deactivated MM (dMM) and non-treated plots (C). Soil samples and roots were assessed in October (before planting), December (2 months after planting), March (early spring) and April (late spring) and known to develop into stalk rot like symptom in late maturing stage, which is not common with this fungus in other part of the world, maybe due to the climate condition unique to the country. In addition to its damage to plant growth promoting rhizobacteria (PGPR) and Paecilomyces lilacinus, the disease severity varied and depended on the Rs strains as well as the plants tested. Strains of phylotype IV, biotype n2 strains. None of the strains belonged to phylotype I, biovar 3 or 4 wilted in pots, only half of them showed similar response in field and pots for the pathogen.

Pythium root rot of corn in Japan; unique symptom climb up the mature stem

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Root rot of corn (Zea mays) caused by Pythium spp. is observed in many part of the world and one of the most important factors of the plant destruction in Japan. It has been recognized long as a disease caused thoroughly by Pyricularia oryzae, and known to develop into stalk rot like symptom in late maturing stage, which is not common with this fungus in other part of the world, maybe due to the climate condition unique to the country. In addition to its damage to plant growth promoting rhizobacteria (PGPR) and Paecilomyces lilacinus, the disease severity varied and depended on the Rs strains as well as the plants tested. Strains of phylotype IV, biotype n2 strains. None of the strains belonged to phylotype I, biovar 3 or 4 wilted in pots, only half of them showed similar response in field and pots for the pathogen.

Effect of soil amendment with seeds of Vernonia anthelmintica on soilborne diseases and growth of okra

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Seeds of Vernonia anthelmintica has great medicinal value and are traditionally used for the treatment of various diseases particularly worms infestation. In this study, water extract of seeds caused significant nematicidal effect by killing 2nd stage juveniles of Meloidogyne javanica in vitro. Soil amendment with Vernonia seeds alone or with Pseudomonas aeruginosa, the plant growth promoting rhizobacteria (PGPR) and Pseudomonas aeruginosa, the egg parasite of root knot and cyst nematode showed significant suppressive effect against root rotting fungi Fusarium solani, F. oxysporum and root knot nematode M. javanica on okra ( Abelmoschus esculentus) roots. In field plot experiment, soil amendment with Vernonia seeds or asafoetida, gum from Ferula asafoetida alone or with PGPR caused similar suppressive effect on soilborne pathogens. Vernonia seeds, asafoetida and PGPR caused positive impact on plant growth by producing taller plants and greater fresh shoot weight. However, mixed application of PGPR with Vernonia seeds or asafoetida resulted in highest yield as compared to other treatments. Soil amendment with Vernonia seeds seems to be a good alternate of chemical pesticides for managing the okra root diseases.

Transcriptional regulation of complementary sense genes in geminiviruses

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Geminiviruses of the genus Begomovirus express a multifunctional protein, AL2, which modulates metabolism, regulates transcription and suppresses RNA silencing. Analysis of promoter-reporter deletions has identified sequences necessary for expression of Tomato golden mosaic virus (TGMV) AL2 in Nicotiana benthamiana protoplasts. Transcription of AL1629 mRNA is dependent on binding of nuclear factors to a 9 bp viral sequence and mutations in the binding site result in a 2 to 6-fold reduction in RNA accumulation. We have isolated a full-length CDNA clone using a 20bp element within the TGMV AL1629 promoter as a target in a yeast one-hybrid
screen. The cDNA encodes a member of the ethylene response transcription factor (ERF) family. ERF proteins can act as transcriptional activators or repressors, and this particular ERF is most closely related to the former. This is consistent with a role in activation of AL6269 promoter. ERFs are ethylene-inducible DNA binding proteins that regulate responses to abiotic and biotic stresses, and have been described in tomato, rice, N. tabacum and Arabidopsis. Of relevance here, is that ethylene can increase either resistance or susceptibility to pathogens. Transgenic plants expressing tobacco (NtERF5) or tomato (Pt4 and Pt5) erf genes show enhanced resistance to Tobacco mosaic virus or Pseudomonas syringae respectively.

Temporal analysis of scab on four passion fruit varieties on brazilian cerrado


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The scab (Cladosporium sp.) is a harmful disease of the passion fruit vine (Passiflora edulis f. flavicarpa). It can occur in all aerial parts of the plant, including leaves, small branches, flowers and fruits. It is a typical young tissue illness causing clorosis in the leaves and can lead to the defoliation of the plant. The aim of this study was to evaluate the progress of the scabon the Gigante Amarelo, Sol do Cerrado, Ouro Vermelho e Rui varieties of passion fruit on brazilian cerrado. Were installed four experimental plots containing three plants of each variety, spaced 2.0 × 4.0 meters in cordon training system. From the flowering were performed 15 evaluations for the disease incidence and severity. The varieties showed no significant differences between the AUPIC and AUSPC, and the differences between the AUSPC and AUPIC were not significant. The monomolecular model fitted better to the incidence and severity progress curves for all varieties and showed the highest coefficients of determination and lower residual mean squares.

Grapevines infected with powdery mildew emit specific volatile organic compounds that can be utilized for pathogen detection

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Plants are known to respond to attack by pathogens with emission of specific volatile organic compounds (VOC). These chemical signals may potentially serve as indicators of the presence of disease, thereby aiding in pathogen detection through non-invasive techniques or electrochemical sensing. Powdery mildew (PM, Erysiphe necator Schwein., of grapevines, Vitis vinifera L.), is a ubiquitous pathogen infecting a valuable crop. In this pathosystem, early detection is difficult but can reduce potential fungicide applications, proving economically and environmentally valuable. Rooted cuttings were inoculated with PM conidia via spore suspension and compared to uninfected cohorts in terms of VOC emissions over the course of three weeks. Each plant was completely sealed within a plastic tube, randomized with respect to treatment, and grown under PM-favorable environmental conditions. Headspace from within each tube was regularly sampled using polydimethylsiloxane-coated stir bars coupled with gas chromatography and time-of-flight mass spectrometry. Detected VOC differed between infected and uninfected plants, between sampling periods, and between infection / sampling period interactions. A description of temporal differences in VOC emission in response to pathogen infection may aid future in situ detection attempts. Specifically, it may be possible to detect indicator VOC in lieu of physical signs of the pathogen at early stages of infection.

The cryptic dimension of host-pathogen interactions: Physiological impacts of Fusarium cinerinctum infection on symptomless Pinus radiata

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Fusarium cinerinctum causes pitch canker, a damaging disease of pines worldwide. A typical symptom of the disease is shoot dieback, but the pathogen can also reside in soil and infect seedlings, which may die or remain symptomless. Nursery-grown trees that sustain latent infections provide a vehicle for pathogen dissemination and are prone to failure after out-planting. For these reasons, it is of interest to better understand the nature of the relationship between the pathogen and the host during the symptomless stage. To this end, we established symptomless infections in Pinus radiata seedlings in order to assess: 1) the impact on plant growth and root system structure, 2) the extent of root rot, 3) the frequency of pathogen isolation, 4) pathogen biomass based on Q-PCR, 5) longevity of the symptomless stage, and 6) impacts on plant response to water stress. Infected seedlings could remain symptomless for over one year. Infected (but symptomless) plants grew 30% faster than control seedlings, roots showed no evidence of rot but overall root system morphology was distinctly altered, with greater mycorrhizal root branching than control seedlings. Under drought conditions, infected plants wilted more rapidly than non-inoculated seedlings, suggesting a predisposition to water stress. This may help to explain a greater mortality risk for transplants that are cryptically infected by F. cinerinctum and suggests greater complexity of pathogen impacts in native forests.

Effect of Puccinia emaculata infection on ethanol production potential of Panicum virgatum

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Rust (Puccinia emaculata) infections have been reported for switchgrass (Panicum virgatum), a model biofuel crop thought to have few disease problems. Infection may reduce biomass and ethanol yield. This study examined the effect of varying degrees of rust infection on switchgrass ethanol yield. Naturally infected leaves from field-grown Alamo and Kanlow in Knoxville, TN were visually categorized as low (LD), medium (MD), or high (HD) disease based on degree of chlorosis, necrosis, and sporulation. Rust was isolated from select leaves to confirm infection. Vegetative (V) tillers were used for LD and reproductive (R) tillers for MD and HD. Leaves from V and R tillers of a disease-free, greenhouse-grown Alamo clone were used as controls. Leaves were dried, ground to 0.063 mm, acid/heat pretreated, and subjected to simultaneous saccharification and fermentation with S. cerevisiae and D. gigantea with two runs per material set. HPLC was used to assess ethanol yield. Ethanol yield differed significantly among disease levels within cultivars and between V and R stages. In run 1, V produced 19% less ethanol than R. MD had 33 and 36% less ethanol, and HD had 54 and 57% less ethanol than LD in Alamo and Kanlow respectively. In run 2, R produced 16% less ethanol than V. MD had 13 and 48% less ethanol, and HD had 26 and 60% less ethanol than LD in Alamo and Kanlow respectively. Ethanol yield loss in rust infected switchgrass may contribute to reduced economic value.

The mixed infections of peach trees by Pseudomonas syringae pathovars in Mazandaran Province, Iran

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Numerous samplings were made from peach trees suffering from old and chronic canker disease. Different suspensions were prepared from leaf spot, canker, root die back and canker, and Alamo leaves to further characterized. According to biolog GN2 kit and persicomycine test three of the isolates were found different from other isolates. These were cultured on both KB and NA media. A complex of bacterial isolates with distinct morphological characteristics were collected during a long run isolation procedures. A total of 30 isolates were selected for further studies. Based on physiological and biochemical characteristics, RRIC–PCR patterns and pathogenicity tests on indicator plants and host seedlings, the most prevalent bacterium was identified as Pseudomonas syringae pv. syringae. Two of the isolates were found different from other isolates. These were further characterized. According to biolog GN2 kit and persicomycine test they were tentatively identified as Pseudomonas syringae pv. persicae. The incidence of the latter is new record for Iran.

Identification and characterization of fungal endophytes from a Greek tall fescue collection

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The Epichloë (Epichloë and Neotyphodium species) are agriculturally important fungal symbionts that associate with cool season grasses. This association is known to confer several benefits to the plant host, including drought resistance and decreased herbivory due to secondary metabolites produced by the fungal partner. A tall fescue collection from Greece was evaluated for the presence of Epichloë to identify and characterize novel endophyte strains. Of the 88 lines investigated, 38 lines were infected with
Neotyphodium species. Pure cultures of each endophyte were obtained from infected tillers. Individual isolates were classified according to morphological characteristics, including growth rate, conidia morphology, and colony appearance. Isolates were also subjected to phylogenetic analyses of the tef1 and tub2 genes and the ITS region. The alkaloïd potential of each isolate was determined using PCR. Results indicate that the Greek isolates are likely to produce some ergot alkaloids and/or indole-diterpenes in planta. Characterization of these secondary metabolism clusters will result in a greater insight and understanding into the evolution of the Epichloë.

Detection and distribution of Root-lesion nematodes (Pratylenchidae) on fruit trees in Northeast regions of Iran

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Root-lesion nematodes are considered as economically important nematodes which cause severe damage to fruit trees. They are always responsible for causing necrotic lesions on the roots. During April-October of 2009 to 2011, a total of 60 soil and root samples of different pome and stone fruits were collected from several gardens in Northeast regions of Iran. Nematodes were extracted from subsamples of 5 g of roots and 250 g of soil with a modification of the sugar centrifugal flotation method. For identification, nematodes were transferred to pure glycerin and mounted on slides. Five species belonging to three genera from Pratylenchidae were identified based on morphological and morphometric characters. Pratylenchoides ritteri was isolated from Mulberry, Walnut, Fig, Apricot, Apple, Nectarine, Orange, Peach, Olive and Hazelnut trees, Pratylenchus neglectus isolated from Walnut, Quince, Plum, Apple and Olive trees, Pratylenchus thornei isolated from Walnut, Quince, Plum, Cherry, Orange, Apple, Persimmon, Nectarine, Hazelnut, Walnut, Fig and Olive trees, Pratylenchus vulnus isolated from Walnut and Fig trees, Zygostylaenus guevrai isolated from Quince and Fig trees. The most prevalent species were Pratylenchoides ritteri and Pratylenchus thornei with high population density through visited regions. Infected plants had roots with dark lesions, a symptom typical of the attacks by these nematodes. To our knowledge, this is the first report of lesion nematodes infecting fruit trees plants in Northeast of Iran.

A species-specific primer for detecting Botryosphaeria dothidea

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Phytopathology 101:S175

Botryosphaeria dothidea can attack a wide range of hosts. It causes apple ring rot, a severe disease in China. The objective of this study is to develop a fast method for detection and quantification of B. dothidea. A pair of primers (ipF and ipR) was designed based on the ITS region of rDNA and a PCR product of 347bp was amplified from DNA extracted from mycelia of B. dothidea, but not from that of Colletotrichum gloeosporioides, Alternaria alternata, Marssonina mali, Monilinia fructigena, Venturia inaequalis, Botrytis cinerea or Valsa sp., the commonly present species in apple orchards, or Botryosphaeria ribis the closely related species. As low as 1pg DNA was detected using conventional PCR as assay. The PCR assay with this specific primer was capable of detecting the pathogen in infected apple fruit tissues. Subsequently, a more sensitive SYBR Green real-time PCR system was developed. The detection limit of the real-time PCR system was estimated as 100fg DNA, which is more efficient and accurate than the conventional PCR. A linear relationship between number of spores counted with compound microscope and the corresponding number of spores determined with the real-time PCR was obtained and as low as 20 spores can be detected. This method may be used in quantifying spores in spore suspensions collected in the field for the purpose of disease monitoring.

Evidence of a low rate of seed transmission of Citrus tatter leaf virus in citrus

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Citrus tatter leaf virus (CTLV) (Apple stem grooving virus) is mechanistically transmitted in citrus, causing bud-union crease in trees budded on trifoliate orange and its hybrids, but is symptomless in most scions. Seed transmission of a strain of CTLV has been reported in Lilium longiflorum and Chenopodium quinoa. In order to test whether CTLV is seed transmitted in citrus, seed was collected from four adjacent CTLV-infected citrus trees of different species: namely Clementine mandarin, Meyer lemon, Eureka lemon and Meiwa kumquat. The resulting 355 seedlings and the four parent trees were tested for CTLV presence by RT-PCR using three primer sets. The four parents and two of the 136 Eureka lemon seedlings, were found to be CTLV positive. This is the first report of CTLV seed transmission in citrus. Cloning and sequencing of the coat protein gene amplified using primer set TL1 showed that the sequence from the seedlings had an 89.7% homology with the parent tree, but no homology with the Meyer lemon and Meiwa kumquat trees, suggesting a filtering effect during transmission through the Eureka lemon parent as reported elsewhere.

Effect of temperature on bacterial leaf spot of Phalaenopsis, caused by Acidovorax castellanii


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Bacterial leaf spot of orchid, caused by Acidovorax castellanii, is common in south Florida nurseries. Little is known about the epidemiology of the disease. The effect of temperature on disease development was investigated using growth chamber studies. Phalaenopsis sp. orchids were wound inoculated with a cell suspension (10⁵ spores/ml) of an isolate of A. castellanii, then placed in humidity chambers at 15, 20, 25, 30, and 35°C with a 12-hour light/dark cycle. There were six inoculated plant replications and two non-inoculated controls at each temperature. Symptoms appeared after 14 days of incubation. Every 7 days, the number of lesions and the percent disease severity was measured for the top three leaves of each plant. At 28 days after inoculation, inoculated plants at 25, 30, and 35°C had significantly more average lesions per leaf than those inoculated at 15°C, which developed bacterial spot symptoms. There was an increase in disease severity at 30 and 35°C compared to lower temperatures. There was a high incidence of naturally infected bacterial soft rot at the higher temperatures. From this data, we conclude that increased temperature increases the severity of bacterial leaf spot of orchid.

Microscopic observation of the interaction between the soybean sudden death syndrome pathogen and soybean cyst nematode, in soybean roots

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Fusarium virguliforme, sudden death syndrome of soybeans (SDS), and Heterodera glycines, the soybean cyst nematode (SCN), impair soybean production in the U.S. Infection by SCN affects severity of SDS symptoms, but the mechanisms behind this interaction are not well understood. Our objectives were to select an optimal inoculation method with both pathogens and to determine if SCN penetration sites serve as entry points for F. virguliforme. Three methods were compared on an SCN- and SDS-susceptible cultivar; infestation of sand-soil mix with SCN females and inoculations of roots with SCN eggs in pouches and in sterile sand. Roots were exposed to SCN from 2 to 20 days and sampled and stained every other day to assay SCN infection. A subset of plants was then inoculated with F. virguliforme and incubated at 27°C for 10 days. Infestation of sand-soil mix with SCN females resulted in more penetrated juveniles per unit root length at each sampling time. The most juveniles were observed in roots inoculated with SCN for 8–10 days. In initial samples, F. virguliforme was found in proximity to SCN juveniles as well as in their absence. Further work is needed to clarify if F. virguliforme colonization is more abundant around SCN infection sites. SDS foliar symptoms were observed in plants inoculated with F. virguliforme after 14–20 days of SCN exposure, but not in the absence of SCN. This suggests that SCN facilitates penetration of F. virguliforme into the root vascular tissue.

Anthracnose disease of Capsicum spp.

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Anthracnose disease of Capsicum annuum (chili) is caused by a complex of Colletotrichum species with C. truncatum, C. acutatum, C. gloeosporioides, being the most severe pathogens. Elucidation of the disease cycle for C. truncatum indicated that seed infection and quiescent leaf infection were important sources of inoculum (2). A convenient, efficient use of integrated disease management practices to prevent fruit infection. Taxonomy of the Colletotrichum spp was validated using three fungal gene sequences (ITS rDNA, partial β-tubulin; translation elongation factor 1-alpha) and species-specific microsatellite markers (STMS). Pathogenicity analysis of C. truncatum/C. capsici isolates collected from various hosts in Australia identified the existence of formae speciales subgroups that were host specific to soybean and custard apple. Differential reactions on maturing green and ripe chili fruit of 10 genotypes of cultivated Capsicum spp identified five, 11 and...
three pathotypes of C. truncatum, C. gloeosporioides and C. acutatum respectively. This will have profound effect on chili breeding programs where novel sources of resistance genes from related species are being incorporated into commercial C. annuum varieties. Putative PR genes have been identified through transcriptional analysis from a virulent pathotype of C. truncatum and an Agrobacterium-mediated fungal transformation system developed for assessing function of these genes.

High throughput screens reveal Salmonella behaviors required for persistence in tomatoes
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The outbreaks of vegetable-associated gastroenteritis suggest that entereic pathogens multiply and persist in plants for extended periods of time, eventually infecting people. The pathways which control how enterics colonize plants, are still poorly understood. To better understand interactions of Salmonella enterica sv. Typhimurium with tomatoes, a collection of deletion mutants and a gfp-tagged Salmonella promoter library were screened inside fruits. Dozens of constructs that were differentially regulated in tomato relative to in vitro growth were identified. The expression of these promoters was tested in planta using recombinase-based in vivo expression technology and fitness of the corresponding mutants was tested. Gene expression in Salmonella was affected by tomato genotype and maturity. For example, a fabH promoter was upregulated in immature tomatoes in response to linoleic acid. The regulation of cryB was activated in the fruit of cv. Hawaii 7997 (resistant toRalstonia solanacearum) more strongly than in the varieties that are more susceptible to Salmonella proliferation. Salmonella motility and animal virulence genes (hilA, fliC, flhDC, fljE and those encoded on the pSLT virulence plasmid) did not contribute significantly to fitness inside tomatoes. Thus Salmonella relies on a distinct set of metabolic and regulatory genes, which are differentially regulated in planta in response to host genotype and fruit maturity.

Response of pepper (Capsicum annuum) genotypes to co-infection by Phytophthora capsici and Meloidogyne incognita
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Phytophthora capsici (PC), the causal agent of Phytophthora blight, and Meloidogyne incognita (MI), the southern root-knot nematode, are both important pathogens of pepper (Capsicum annuum L.) in the U.S. We studied the responses of five pepper genotypes with differing resistances to PC and MI to co-infection by both pathogens, with and without mandipropamid and oxamyl, in greenhouse tests. Pepper genotypes were CM-334, a serrano-type; RD (moderately high resistance to PC); Aristotle (moderately resistant to PC); and Jupiter (PC susceptible). CM-334 and Charleston Belle exhibited high resistance to MI (root galling range = 0% and 1%, respectively). Jupiter, Aristotle, and RD were susceptible to MI (root galling range: 45% to 68%). Oxamyl treatment was effective in controlling MI in the bell genotypes (root galling range: 0% to 2%). CM-334 was resistant to PC with 7% root rot and 12% crop damage. All bell peppers were susceptible to PC (root necrosis range: 30% to 33% and crown necrosis range: 50% to 65%). Bell peppers treated with mandipropamid had significantly less (P = 0.0165) crown necrosis than non-treated plants (23% vs. 58%). Inoculation with MI did not affect Phytophthora blight symptom development in any genotypes. The results in this study indicate that MI does not have a significant effect on predisposing PC-resistant or susceptible pepper genotypes to Phytophthora blight.

Pre- and post-anthesis activity of fenbuconazole and triforine against blueberry flower infection by Monilinia vaccinii-corymbosi
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Monilinia vaccinii-corymbosi infects open blueberry flowers via stigma and style, causing reproductive disease. Management of flower infection relies on multiple fungicide applications based on bloom progression. Since bloom can be highly protracted, however, flowers that open between applications remain prone to infection because the stigmatic surface is not exposed to active ingredient – unless there is systemic movement in the pistil and/or residual activity in the ovary. In greenhouse experiments with fungicide applications at defined flower stages and conidial inoculations conducted 1 day after anthesis, the systemic fungicides fenbuconazole and triforine provided excellent protection to newly opened flowers sprayed at anthesis, but differed in their pre-anthesis activity in unopened flowers. Fenbuconazole suppressed infection in unopened flowers treated 2.5 days pre-anthesis, whereas triforine provided substantial protection to earlier flower stages up to 7 days pre-anthesis. In a separate experiment to quantify post-infection activity, fenbuconazole and triforine significantly suppressed infection when applied within 2 and 6 days after anthesis, respectively. Thus, the total window of activity of fenbuconazole is only 4.5 days, whereas that of triforine is up to 13 days. To better manage flower infection by M. vaccinii-corymbosi, active ingredients such as triforine with superior systemic and/or residual activity in flowers need to be identified.

IR-4 Project fungicide registration on specialty crops update
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In 2010 and early 2011, the IR-4 Project obtained new uses of 5 fungicides on many specialty crops with a total of 147 new fungicide uses being registered through U.S. EPA. New fungicide registrations on food crops included cyazofamid, difenoconazole, flutolanil, fluzinam, and mancozeb. Azoxystrobin, fludioxonil, and difenoconazole were submitted for post-harvest use on potato to control silver scurf and Fusarium dry rot. IR-4 is developing data to verify that bacterial spot of pepper is controlled with low rates of acibenzolar without yield loss. Cyazofamid was submitted for use on basil downy mildew. Switch (cyprodinil + fludioxonil) was submitted for anthracnose control on pepper and spinach. Acibenzolar was submitted for use on strawberries to control angular leafspot. Metconazole was submitted for use on potatoes to control early blight. Mancozeb was labeled on additional tropical fruit, ginseng, and the entire cucurbit crop group. Cyazofamid was labeled on brussica crops for downy mildew and club root control as well as downy mildew control on turnip greens, spinach, and hops, and white rust of spinach. Fluzinam was labeled on onions and lettuce for control of a number of diseases. Flutolanil was labeled for use on brassica crops for control of wiltstem caused by Rhizoctonia spp. Two developing crops, dragon-fruit (Pitaya) and wasabi, will soon obtain registrations of a number of fungicides: L. chlorothalonil, azoxystrobin and Switch on dragon-fruit, and azoxystrobin on wasabi.

Tradeoffs between host adaptation and vector transmission of Soybean Dwarf Virus
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New emergent viruses must surmount critical limitations for transmission, infection, and replication in order to expand their host range. To evaluate the risk of indigenous clover strains of Soybean Dwarf Virus (ShDV) as an emerging plant pathogen threatening soybean production in the United States, we compared Sequence Type ShDV-MD6 was serially transmitted from clover to soybean and pea by aphid vectors, Neartaphis bakeri and Acrystosiphon pisoni, respectively. Genomic RNA sequence analysis of ShDV-MD6 following passages on soybean and pea identified 11 non-synonymous consistent mutations in soybean, and 4 mutations in pea. The d0/d1 analysis indicated positive selection pressure on MD6 in soybean, but not in pea. Significantly increased virulence of virus titers with each sequential transmission support this analysis. In the soybean line, virus titer in the third passage was approximately 100 times more than the first passage. However, transmission efficiency on soybean decreased with each passage from 53% to 0%, until the virus was no longer aphid transmissible. The level of intra-host genetic diversity of virus populations in a single infected plant increased from 0.08% on average to 0.11% after reaching equilibrium in the new host. Results indicated that the clover strain of ShDV-MD6 adapted readily to soybean by improved replication and/or movement, but selection for host adaptation created tradeoff factors decreasing host-to-host transmissibility by aphid vectors.

Study on molecular targets of hydrogen peroxide in fungal pathogen mitochondria under oxidative stress
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We mainly investigate the mechanistic process at the proteomic level in Penicillium expansum, by identifying the molecular targets of H2O2 that is frequently used as a response of the host cells. Then, we performed mitochondrial sub-proteomic analysis to seek the molecular targets of H2O2. A set of mitochondrial proteins were identified, including respiratory chain complexes I and III, F1F0 ATP synthase, and mitochondrial phosphate carrier protein. The functions of selected proteins were further investigated to determine their effects on the H2O2-induced cell death. Through fluorescent
co-localization and the use of specific inhibitor, we provide evidences that complex III of the mitochondrial respiratory chain contributes to ROS generation in mitochondria under H2O2 stress. The undesirable accumulation of ROS caused oxidative damage of mitochondrial proteins and led to the collapse of mitochondrial membrane potential. Additionally, we prove that ATP synthase was involved in the response of fungal pathogen to oxidative stress, because inhibition of ATP synthase by oligomycin decreased cell survival. The results suggest that mitochondrial impairment due to functional alteration of specific proteins is associated with cell death of fungal pathogen caused by H2O2. The identification of mitochondrial targets can provide a basis for future development of novel antifungal agents.

Effect of environmental conditions and lesion age on sporulation of *Phytophthora ramorum* on California bay, rhododendron, and camellia

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*Phytophthora ramorum* is the causal agent of the disease known as Sudden Oak Death (SOD). The objective of our research was to determine the environmental conditions and lesion age favorable for *Phytophthora ramorum* sporulation under field conditions. For two years, new camellia, rhododendron, California bay (Umbellaria californica) nursery stock were seasonally inoculated (every 3 months) on foliage. They were covered overhead to prevent rainfall from falling on the plants, but otherwise the plants were completely open to the natural environment. Consistent leaf wetness periods were produced with overhead misting systems and controlling sensors to simulate rainfall, fog, dew, or other conditions that might be supportive of sporulation in an irrigated nursery or landscape. For each season, these wetness conditions began when leaf lesions were 3, 6 and 9 weeks old and, at each of these time points, the wetness conditions were maintained for 8 days. Sporulation was evaluated by washing leaf lesions before the wet period began and at 1, 2, 4 and 8 days during the wet period. Leaf wetness and temperature were measured near the plants. For rhododendron, a Poisson regression model demonstrated that sporulation increased with increasing lesion age. Sporulation increased with increasing consecutive hours of leaf wetness up to about 48 hours. Sporulation decreased with higher maximum temperatures. Sporulation often occurred when leaves were coated with naturally forming dew.

Characterization of *Pythium nunn* newly recorded in Japan on antagonistic activity against *P. ultimum* and *P. aphanidermatum*

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*Pythium nunn* is a potential biocontrol agent first recorded from a grassland soil in Colorado, U.S.A. This species, which has never been recorded as a plant pathogen, suppresses several soilborne diseases, was also nunn was recently first reported in Japan and was characterized by its antagonistic activity against *P. ultimum* var. *ultimum* and *P. aphanidermatum*. Three *P. nunn* isolates were obtained from soils in Nagano, Osaka and Fukuoka prefectures. The morphology of all isolates corresponded with those of the original description of *P. nunn*. The ITD-ITS sequences of the three isolates were identical in sequence and had a higher similarity with the sequences of the type strain of *P. nunn*. The *P. nunn* isolates were mycophagous toward *P. ultimum* var. *ultimum* and *P. aphanidermatum*. They suppressed damping off of cucumber caused by *P. ultimum* var. *ultimum* and damping off of creeping bentgrass caused by *P. aphanidermatum* at an early growth stage of the plants.

Effect of barley chromosome addition to wheat on the preference and performance of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae)

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The migratory locust *Locusta migratoria* exhibits density-dependent phase alternation and is potentially one of the most destructive agricultural pests worldwide. The locust feeds on various Poaceae such as wheat, but does not eat barley. Identification of the barley genes inhibiting feeding by the locust is useful for developing resistant crops. Using six barley chromosome disomic addition lines of wheat (2H-7H), we investigate the effects of barley chromosome addition to wheat on the preference and performance of *L. migratoria*. The locomotor activities of hatchlings given seedlings of wheat and 2H-7H were not significantly different one another, but lower than those of hatchlings given barley. Feeding preference by locust hatchlings was investigated by choice tests in which hatchlings were given wheat and one of the chromosome addition lines for 24 hours. The mean amount of wheat consumed by hatchlings was significantly larger than that of 2H, 5H, and 6H consumed. Growth performance of the hatchlings fed with wheat, barley, or 2H-7H was assessed by examining their survival rate and developmental period. The duration of the first instar for hatchlings given 2H or barley was significantly prolonged than that for those given wheat or other addition lines. These results suggest that the barley genes involved in the inhibition of feeding by locusts exist on barley chromosomes 2, 5, and 6, and those causing slow development of the locust on barley chromosome 2.

From bacteriosi of the fall webworm *Hyphantria cunea* to development of bio-insecticide based on * Bacillus thuringiensis* in Kazakhstan

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The fall webworm (*Hyphantria cunea* Drury) is a quarantine pest for Kazakhstan. Its mass invasion of tree plantations was discovered in 2003 in southeast Kazakhstan. In 2004 its nests with dead larvae were found on a maple tree (*Acer negundo* L.) with sign of bacterial infection. Septicemia was caused by crystal-forming bacteria *Bacillus thuringiensis* var. *kurstaki*. Strain 2127-3K with high production of parasporal endotoxin-crystals was derived from the R-form of *B. thuringiensis*. The mortality of the larvae after infection with *B. thuringiensis* strain 2127-3K was 3.8 times higher than with strain Z-52 of the Russian commercial bio-insecticide “Lepidocide” used as a control. Insecticidal activity based on *B. thuringiensis* strain 2127-3K, the first local bio-insecticide “Ak Kobelek” was registered in Kazakhstan against a broad range of Lepidopteran pests.

Host specificity of *Cochliobolus* sp., a new pathogen of warm-season turfgrasses

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A new foliar disease was initially observed on bermudagrass (*Cynodon dactylon*) and zoysiagrass (*Zosia japonica*) putting greens and fairways at golf courses in the Houston, Texas area in 2007. Disease symptoms on individual leaves exhibited prominent, black, elliptical lesions along the leaf margins. Symptoms on the closely mowed turfgrass appeared as dark, brownish-black spots 5-cm in diameter. Isolates of *Cochliobolus* sp. were consistently recovered from field samples and Koch’s postulates were conducted to confirm pathogenicity. A greenhouse study was conducted to determine the host range of these isolates. Surface disinfested seed of bermudagrass, zoysiagrass, centipedegrass (*Eremochloa ophiourhoides*), and seashore paspalum (*Paspalum vaginatum*) were sown into *Cochliobolus*-infested soil contained in 5-cm pots. St. Augustingrass (*Suntanuprum sandwicense*) and fescue were also tested as a possible host. Disease severity was greatest in zoysiagrass which appeared scorched or desiccated. Leaf symptoms consisted of elliptical lesions with gray, necrotic centers and black margins. Bermudagrass and St. Augustingrass developed foliar lesions but severity was low. Centipedegrass and seashore paspalum only had a reduction in seedling establishment. *Cochliobolus* sp. was re-isolated from all hosts tested but not from seashore paspalum. The fungus did not cause root discoloration in any of the hosts indicating that it is only pathogenic on foliar tissues of select warm-season turfgrasses.

Efficacy of spring fenarimol applications for spring dead spot control in a Tifway bermudagrass fairway in Mississippi

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Spring dead spot, caused by *Ophiophaeella korrae*, is an annual disease of bermudagrasses that undergo dormancy. A study was conducted on a golf course with a Tifway bermudagrass fairway in West Point, MS to evaluate the effectiveness of spring fenarimol fungicide treatments and fertilizer for controlling this disease. Treatments served as the main plot factor and applied fenarimol (spring, fall, spring followed by fall), propiconazole, myclobutanil, and azoxystrobin (all fall), and a check. Fertilizer nitrogen source (sub-plot factor) was applied as an organic or inorganic (ammonium sulfate) 12N-2P-12K at 0.5 N kg per 93 m2. Spring dead spot severity and percent disease were visually assessed in April, 2009 and 2010. Turfgrass quality was rated monthly and spring green-up was recorded in March of both years. The fenarimol, spring only treatment, was equally effective, based on SDS severity, as fall treatments of fenarimol, propiconazole, myclobutanil, and azoxystrobin. The nitrogen source did not influence spring dead spot control. Turfgrass quality and spring green-up were similar for all treatments.
An advantage of a spring fungicide application is that only areas where the disease is active and exhibiting SDS symptoms are treated. This allows the superintendent to make sight-specific fungicide applications on fairways that may in turn be more cost effective as well as provide an acceptable level of spring dead spot control.

Micro-biota associated with wild and cultivated strawberry and their potential use as biological control agents for strawberry black root rot

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Black Root Rot (BRR) is a disease complex on strawberry conferred by one or more organisms including Pythium, Fusarium, Rhizoctonia spp. and several species of nematodes. Eighty percent of strawberry acreage is pre-plant fumigated with methyl bromide. The pending elimination of MB use concerns strawberry growers and has stimulated the search and evaluation of ecologically-based strategies for the management of BRR. Project objectives are to survey, isolate and identify microorganisms present in the rhizosphere and endosphere of wild (Fragaria virginiana) and cultivated strawberry (F. x ananassa). Microbiological and molecular techniques were used to determine the microbiota present in roots, crowns and rhizopoeic soil of 5 different populations of strawberry (2 nurseries, 2 wild and 1 organic system). The diversity of pathogenic fungi as well as bacteria and fungi populations differed in each plant source. The average CFU gr-1 dry soil of Pythium was 288 on cultivated strawberry soil, versus 2268 on wild strawberry’s. For Rhizoctonia, 6281 on cultivated versus 11602 on wild. For general fungi, 37543 on cultivated and 172810 on wild strawberry soil. Despite of this, no disease symptoms were found on wild strawberries. Over 150 isolates have been obtained including potential beneficial Trichoderma spp. and Paecilomyces lilacinus. Further evaluations will be made to determine the role of this and other beneficials and their use in an integrated disease management system for strawberry BRR management.

Clonal and sexual dispersal of Armorillaria mellea in an ornamental landscape


Phytopathology 101:S178

High densities of planted hosts and frequent irrigation have contributed to severe Armorillaria root disease in Golden Gate Park, San Francisco, CA. Our objective was to assess the relative contribution of vegetative growth and basidiospore dispersal to the colonization of the park by Armorillaria mellea. We investigated the genetic structure of A. mellea at a fine spatial scale using microsatellite data. Ninety-five unique multilocus genotypes were identified among 166 isolates. Only 28 genotypes (29%) were shared by two or more isolates (clones). The largest two clones, resulting from vegetative growth of one genotype, measured 216 m and 322 m. Spatial autocorrelations of kinship coefficients, with and without clones, converged at an average distance of 130 m, indicating that this distance constitutes the linear spatial dimension above which clonality does not affect the genetic structure of the population. Up to 100 m, genetic similarity between pairs of isolates decreased systematically in a linear space (SAR) response. Base and 100 m, a random spatial distribution of genotypes was observed, consistent with an establishment from sexual spores from distant sources. The absence of multilocus linkage disequilibrium and the high proportions of genotypes detected only once suggest that most infections in the park resulted from basidiospores. However, 29% of genotypes infected multiple trees as a result of subterranean, vegetative growth.

Use of Datura stramonium and Nicotiana benthamiana to study Acanthosar-S-Methyl-induced SAR against Iris yellow spot virus (genus Tospovirus)

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Acanthosar-S-Methyl (ASM) is a functional analog of salicylic acid (SA) that activates a SAR-like system in several plants. SAR-mediated resistance (SAR) response against a wide variety of pathogens including bacteria, fungi and viruses. ASM has been shown to induce SAR against Tomato spotted wilt virus (TSWV) in Nicotiana tabacum and other hosts. Iris yellow spot virus (IYSV) is an economically important tospovirus of onion. We are using Datura stramonium and tobacco Nicotiana benthamiana as model plants to develop a set of descriptors to study the effect of SAR inducers on IYSV-host interactions. ASM- and buffer-treated D. stramonium and N. benthamiana plants were mechanically inoculated with IYSV. Symptom development and virus levels were monitored. After 10–14 days post inoculation, leaves were tested for IYSV by ELISA. Significant reduction in virus levels in the ASM-treated plants was noticed. The ELISA results were confirmed by RT-PCR. The level of SAR response was assessed by measuring the lesion sizes on the systemically infected leaves of the ASM-treated plants. ASM-treated plants showed reduced viral symptoms compared to buffer-treated plants. Our results suggest that both V. benthamiana and D. stramonium can be used efficiently to study the effect of SAR inducers on IYSV. These model plant systems also facilitate studies to quantify the levels of various IYSV genes during the ASM-induced plant defense response.

Adapting synthetic gene circuits for plant-based detection of pathogen indicators: A test case


Phytopathology 101:S178

Recently the principles of synthetic biology were applied to the development of transgenic detector plants that visibly de-green or turn white in the presence of specific chemical compounds. The strategy required three components: a receptor that relays a signal in the presence of a chemical input, a response-relay protein that carries the signal to the nucleus, and genes activated in response to the signal (i.e., encoding a de-greening response gene circuit or luciferase reporters). We are adapting this strategy to develop the first generation of plants that detect and respond to small molecules secreted by bacterial plant pathogens. Xylella fastidiosa, a devastating quarantine pathogen of citrus and grapes, secretes a small fatty acid diffusible signal factor (XF-DSF). We designed a synthetic receptor that triggers the response relay circuit in the presence of XF-DSF. In a bacterial reporter system, the synthetic DSF receptor elicited a measurable response to nanomolar concentrations of a synthetic XF-DSF analogue, as well as to crude extracts of X. fastidiosa culture supernatants. The receptor is more responsive to fatty acid signals made by K. fastidiosus than those of Xanthomonas species. Arabidopsis lines expressing the receptor exhibited an increase in reporter gene activity in response to infiltration with DSF. These efforts represent the first steps toward the application of plant synthetic biology to detection of pathogens.

Effect of Huanglongbing on the structure and functional diversity of microbial communities associated with citrus rhizosphere


Phytopathology 101:S178

The diversity and stability of the rhizosphere bacterial community heavily influence plant productivity and ecosystem sustainability. The goal of the study was to understand the influence of ‘Candidateis Liberibacter asiaticus’ (known to cause Huanglongbing, HLB) on the structure and functional potential of microbial communities associated with citrus rhizosphere. The results of clone library sequencing and quantitative real-time PCR revealed that ‘Ca. L. asiaticus’ infection re-structured the native microbial community of citrus rhizosphere. GeoChip 3.0 used to determine the effect of ‘Ca. L. asiaticus’ infection on the functional diversity of rhizosphere microbial communities showed that HLB disease has significant effects on various functional guilds of bacteria. Many genes involved in key ecological processes such as nitrogen cycling, carbon fixation, phosphorus utilization were significantly greater in healthy as compared to HLB diseased citrus rhizosphere. HLB infection also caused shifts in the carbon utilization patterns of rhizosphere microbial community. Overall our study provides evidence that change in plant physiology mediated by ‘Ca. L. asiaticus’ infection could elicit shifts in the composition and functional potential of rhizosphere microbial communities. Our results indicate that plant diseases not only affect plant productivity but also cause disturbances in ecosystem equilibrium.

Effect of pre-sowing soil incorporated treatments on Alternaria radicina in carrot Daucus carota

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Alternaria radicina, a seed- and soil-borne pathogen of carrot can cause problems for carrot seed producers. As the pathogen is present in New Zealand soils, the pre-sowing soil incorporation of potential control products
was investigated in a glasshouse study. Four fungicides (difenconazole, difenoconazole + chlorothalonil, iprodione and pyraclostrobin), three fumigants (formaldehyde, dichloropropene + chloropicrin, and metam sodium) and two biocontrol products (Trichoderma atroviride and T. harzianum) were incorporated at recommended label rates into soil which contained 250 CFUs/g of the pathogen. Two carrot varieties were sown 15 days later and emergence recorded at 15 days after sowing. A. radicina soil population density was determined at 0, 4, 16 and 32 weeks after application using a soil dilution plating method. Carrot emergence did not differ between the varieties, and emergence for all fungicide treatments and formaldehyde did not differ from that of the uninoculated control. T. atroviride significantly increased emergence over the inoculated control. All chemical treatments had reduced the A. radicina soil population to between 10 and 100 CFUs/g by 4WAA, and by 32WAA they were between 50 and 150 CFUs/g depending on the treatment. The biocontrol products took 16 weeks to significantly reduce the pathogen population (to ca. 180 CFUs/g) and by 32 weeks had reduced it to ca. 150 CFUs/g. Whether this reduction would have continued is worthy of further investigation, as is field validation.

Calosphaeria canker of sweet cherry in California

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California is the second largest sweet cherry producer in the United States, with annual revenues up to $200 million. Canker diseases and associated branch dieback are responsible for extensive damage throughout California’s sweet cherry orchards, reducing annual yields and tree longevity. Recent surveys and isolation work identified Calosphaeria pulchella, Eutypa lata and Leucostoma persoonii (Syn: Cytospora leucostoma) as the main fungi associated with branch dieback of sweet cherry in California. The most prevalent pathogen, C. pulchella, was isolated from nearly all of the surveyed orchards. Pathogenicity studies showed that C. pulchella operates as a primary pathogen of sweet cherry, infecting healthy tissue through wounds and subsequently causing cankers in the wood. Spore trapping studies conducted in two orchards near Davis and Linden showed that rain as well as sprinkler irrigation water were important factors for aerial dissemination of the ascospores of C. pulchella, which constitute the main inoculum. Plant stress provoked by excessive irrigation and extensive pruning are suspected to contribute factors that contribute to the expression of the disease. Pruning of cherry trees during summer when inoculum availability is low is combined with irrigation practices which do not wet trees should diminish the risk of pruning wound infections with C. pulchella.

Diversity and population dynamics of Xanthomonas axonopodis pv. manihotis in Colombia from 2008 to 2010

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Xanthomonas axonopodis pv manihotis (Xam) is the causal agent of cassava bacterial blight and is one of the most important bacterial problem in this crop. In Colombia, processes of pathogen migration were detected between regions in the 90’s with a concomitant high diversity index. With the purpose of characterizing the current population structure of the pathogen, sampling collections were carried out from September of 2008 until November 2010. Bacterial isolates were characterized using AFLPs then clustering analysis allowed to identify geographical distribution patterns. Additionally, genes coding for the Type Three Effector (T3E) were sequenced to establish their degree of variability and to assess the presence and nature of selection exerted by the host. The results confirmed a prominent diversity of the pathogen on the Caribbean coast and haplotype migration through time. On the other hand, a low variation was detected on the T3E genes, possibly indicating their importance in pathogenesis. However, additional T3E genes have been sequenced in order to confirm these observations. This study shows the current condition of populations of Xam in Colombia and it will contribute to the generation of measurements to manage bacterial blight.

Survey and screening of classical biological control agents for Japanese knotweed (Fallopia japonica)

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Japanese knotweed (Fallopia japonica, Polygonaceae) is a serious invasive weed in the UK, North America and large parts of Europe where there is an urgent need for classical biological control (CBC) strategy. Surveys have confirmed the absence of any significant natural enemy pressure in the UK, and the presence of an extensive guild of specialized natural enemies in the native range of Japan. Among these, although a psyllid, Aphisalara itadori, has been approved ahead for release in the UK, the plant pathogens still remain as potential CBC agents for use against F. japonica. The results of preliminary surveys showed that three fungal diseases of two rusts and a Mycosphaerella leaf spot were predominantly common and widespread in the fields. These rusts were identified as Puccinia polygoni-amphibii var. tovariae and Aecidium polygoni-cuspidati, and confirmed their severe infection to F. japonica both in the field and laboratory studies. However, it suggested that they were heterocercous rusts pathogenic to the non-target species, indicating to be eliminated from the prior agents of CBC ones. On the other hand, a leaf spot fungus morphologically identified as Mycosphaerella polygoni-cuspidati caused severe damaging disease of F. japonica. Additionally, endophytic fungi associated with M. polygoni-cuspidati promoted the disease severity. In conclusion, developments of new strategy for integrated CBC of invasive weeds is suggested.

Effect of fungicide programs on white rot of garlic in central California

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Sclerotina of Sclerotium cepivorum, the fungus that causes white rot in onion and garlic, are capable of survival in the soil for decades. In Central California production areas, once the disease is detected, the field is no longer used for production of allium crops, and at least 5,900 ha are currently infested. Adoption of drip irrigation in these crops presents an opportunity for application of pest control materials. In this study conducted in a naturally infested field in western Fresno County California, fungicide programs include applications in the planting furrow and injected into the drip irrigation system were evaluated from 2008 to 2010. Treatments consisted of 2 to 4 applications through the drip irrigation system. Materials applied first were either tebuconazole or fludioxonil, rotated with the other followed by 1 or 2 applications of boscalid. In subplots, the same three materials were tested as applied immediately before planting into the trench. As compared to non-treated controls, the at-planting applications reduced disease levels by an average of 30, 86 and 42 percent, in 2008, 2009 and 2010, respectively. There were no differences detected between the 3 fungicides tested. Drip irrigation applications yielded no benefit as compared to the untreated control. Potential of 3 fungicides in managing white rot when applied at planting were documented in central California, but drip applications were consistently ineffective.

Projected distribution and severity of clubroot of canola in the Canadian prairies

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Clubroot of canola, caused by Plasmodiophora brassicae Woronin, was first reported in the St. Albert region of Alberta, Canada in 2003 and has subsequently appeared over a broad area around Edmonton, AB. The simulation program CLIMEX® was used to model potential distribution and severity of clubroot of canola in the Canadian prairie region under: 1) current climate using long-term climate normal data (LCND); 2) incremental temperature and precipitation scenarios; and 3) an irrigation scenario. Initial projections of clubroot based on LCND were consistent with observations on cruciferous vegetables in the lower mainland of British Columbia and central Canada, and canola in Alberta. The model suggested clubroot could affect canola over a wide area of the prairies, especially wetter regions. Incremental temperature increases of 1 to 3°C resulted in an expansion of the area potentially affected by clubroot. A scenario of 120% of normal rainfall during the growing season resulted in greater projected clubroot distribution and severity, compared to incremental temperature increases. Incremental decreases in rainfall resulted in substantial reductions in the projected distribution and severity of clubroot. Irrigation may compensate for dryer conditions in the southern Alberta canola growing region of Alberta, resulting in the development of a proactive approach to extension and research is recommended for projected ‘at risk areas’ to limit the potential impact of clubroot on dryland and irrigated canola.
The impact of fungicide and herbicide timing on barley leaf disease severity, weed management and crop productivity

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Phytopathology 101:S180

Field trials were conducted in 2010 to determine the efficacy of tank mixing herbicides and fungicides on leaf disease and weed management, and barley grain yield. Combinations of the herbicide Axial® (pinoxaden) and the fungicide Tilt® (propiconazole) were applied to matling barley cv. AC Metcalfe at the 2-3 leaf stage, 5-6 leaf stage, or the flag leaf stage at three experimental sites (Lacombe, AB, and Melfort and Scott, SK). Prior to seeding of the barley, the plot area was cross-seeded with tame oat as a model weed. Penultimate leaf samples were collected for assessment of leaf disease severity, while weed biomass was also assessed. Plots were harvested and grain yield and kernel quality assessed. Penultimate leaf disease severity was significantly higher for the 2-3 or 5-6 leaf stage no fungicide, herbicide only treatments and the combination herbicide and half rate fungicide treatments at the 2-3 or 5-6 leaf stage compared to all other treatments. Yield and thousand kernel weight tended to be highest for those treatments where the fungicide treatment included a flag leaf stage application. Model weed biomass was very low and generally not influenced by the treatments due to effective herbicide applications at each of the sites. Preliminary results suggest that for malt barley, fungicide applications should include a flag leaf stage timing to ensure control of early blight and tuber and stem blight also contributing to enhanced yields and grain filling. The experiment will continue for two more years.

Prevalence and aggressiveness of Alternaria solani and A. alternata on potato in the Columbia Basin of the Pacific Northwest

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Early blight and brown spot on potato are caused by different species of Alternaria. A. solani, a well-documented pathogen on potato and the cause of early blight, forms tan-colored lesions that have large concentric rings. Lesions of brown spot, caused by A. alternata, tend to be smaller and darker in color, but can be numerous on a leaf. A. alternata was not described as a pathogen on potato until the 1980s. In 2009, 214 isolates of Alternaria were isolated from leaves with lesions from 22 potato fields in the Columbia Basin. In 2010, 163 isolates of Alternaria were collected from 20 fields. The average frequency of isolation for A. solani vs. A. alternata between 6/09 and 8/09 was 9% and 1%, respectively, but after 8/25/09 it was 15% vs. 71%, respectively. In 2010, A. solani was isolated less frequently (18%) than A. alternata (75%) throughout the season except on 7/3/10. Pathogenicity and aggressiveness assays using 34 isolates of both species collected in 2009 were compared. In 2010, A. solani was isolated less frequently (18%) than A. alternata (75%) throughout the season except on 7/3/10. Pathogenicity and aggressiveness assays using 34 isolates of both species collected in 2009 were performed on detached Russet Norkotah leaves. All 17 isolates of A. solani (100%) resulted in lesions whereas 53% (9 of 17) isolates of A. alternata caused lesions. Early blight lesions enlarged more rapidly than brown spot lesions. Lower aggressiveness on potato by A. solani. The differences in prevalence and aggressiveness of these two pathogens will likely impact the timing of their management in the region.

The use of arthrospore formulation of antagonistic Streptomyces for the control of diseases caused by Phytophthora species

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The diseases caused by Phytophthora spp. have been long time serious problem for the cultivation of various important crops worldwide. For the disease, the antagonistic Streptomyces strains with superior competence of sporulation in submerged culture were isolated. An intellectual proprietary pilot scale (up to 750L) fermentation technology which yield well suspended arthrospore formulation at more than 10^{10} cfu/mL was established. The effectiveness of the attempted biofungicide on disease control was demonstrated on late blight of tomato caused by Phytophthora infestans as well as foot rot and gummosis of citrus caused by P. palmivora. The success of disease control depends greatly upon mycoparasitism of the biocontrol agent. For field grown tomato spray treated with the biocontrol agent, the mycelia and zoosporangia of P. infestans on the existing lesions were parasitized and killed; and the expansion and spread of the lesion was inhibited. Likewise, for field grown citrus shown declined growth and gummosis symptoms typical of Phytophthora infection, a coverage treatment with cellulosic nonwoven tape pre-soaked with the arthrospore formulation resulted in healing up of the infected tissue and restored growth vigor. Accumulated evidence indicated clearly great potential for the use of antagonistic Streptomyces as microbial biofungicide for the control of diseases caused by Phytophthora species.

Distribution, pathogenicity, and molecular analysis of Puccinia psidii in Hawaii

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Ohia rust caused by Puccinia psidii was first discovered on the island of Oahu in 2005 and is now found infecting Myrtaceae on all of the major Hawaiian Islands. The genus of susceptible hosts include five Syzygium, five Eugenia species, including Eugenia koollaeensis (nino), four Metrosideros, and one each from genus Callistemon, Chamelaucium, Melaleuca, Myrticia, Myrtus, Pimenta, and Rhodomyrtus. In pathogenicity tests, M. polymorpha, E. koollaeensis, S. jambo, S. samarangense, and P. dioica, all react to Puccinia psidii spores from S. jambo in the same severe manner. Plants are nearly killed with many urediniospores. On a few species, visual variation can be attributed to the host. This is seen for S. paniculatum and R. tomentosa. Molecular analysis of urediniospores collected from various hosts indicate that of the 15 P. psidii genotypes, the rust found in Hawaii is a single strain and is similar to one found in Florida and from an insect of infected myrtle from California.

Dissemination, incidence and severity of Leifsonia xyli subsp. xyli in sugarcane of Sao Paulo state, Brazil

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Sugarcane is the third most important cash crop in Brazil with 8.03 million hectares and production of 624.99 million ton. Despite its importance, research on sugarcane pathogene stands far behind. Little information is available on ratoon stunting disease (RSD), caused by Leifsonia xyli subsp xyli (Lxx), the most important disease of sugarcane worldwide. Therefore, the objective of the present work was to examine the dissemination, incidence and severity of Lxx among sugarcane varieties in 2009 and 2010. Sap of 100 stalks from each field was sent by mills to the laboratory for a routine RSD analyses which allowed examining the incidence. The presence of Lxx was checked by the “dot blot immunoassay” which identified different bacterial populations by the blue dots of different gradations which permitted examining severity. The present work analyzed 187 fields from 35 varieties in 2009 and found Lxx disseminated in 23% of fields in 21 varieties whereas in 2010 from 166 fields and 33 varieties dissemination was 26.5% in 20 varieties. In total 30 out of 49 varieties showed presence of Lxx. Incidence of Lxx in three of the most planted varieties (44.9%) varied from 1 to 72% in RB667515, from 1 to 25% in SP81-3250 and from 1 to 56% in RB855453. Some fields of these varieties showed severity enough to indicate susceptibility to main Brazilian varieties to RSD.

The status of grapevine trunk diseases in British Columbia

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Grapevines (Vitis vinifera L.) is one of British Columbia’s emerging crops with vineyards becoming established in different regions including, the southern interior, southwestern region, and pacific islands. Nowadays, British Columbia wine industry comprises over 5,000 ha representing an economic impact that exceeds $45 million. Grapevine trunk diseases have recently become a major concern among growers. However, the importance that grapevine trunk diseases have on grapevine health in British Columbia has not yet been evaluated. Therefore, field surveys were conducted throughout the main grape-growing regions and diseased samples showing characteristic dieback symptoms including, central vascular necrosis, dark streaking of the wood, light-brown wood discoloration, and perennial cankers were collected from both young and mature vines in British Columbia. Morphological characteristics along with combined multi-allelic DNA sequence analyses from the rDNA (ITS5-ITS2), β-tubulin and EF-1α genes allowed us to identify several fungal species in the families Botryosphaeriaceae, Calosphaeriaceae, Diatrypeaceae, Nectriaceae, Valsaecae, as well as species in the genera Cadophora, Phaeomoniella, Truncateilia, and Pestalotiopsis associated with the different vascular symptoms. This study reveals for first time the presence of grapevine trunk diseases in British Columbia including, black foot disease, Botryosphaeria canker, Eutypa dieback, esca, and young vine decline.
Production of both carboxy-cerominal coat protein forms of Lolium latent virus is required for efficient systemic movement


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The Lolium latent virus (LoLV, Lolavirus, Alphaflexiviridae) genome is encapsidated by equimolar amounts of carboxy-cerominal coat protein (CP) variants of apparent MW 33 and 28 kDa. The CP ORF contains two 5’-proximal AUGs, encoding Met 1 and Met 49, respectively promoting translation of the 33 kDa and 28 kDa CP variants. The 33 kDa CP N-terminal domain includes a 42 aa sequence encoding a putative chloroplast Transit Peptide (cTP) with a predicted cleavage site upstream of AUG2. Ablation of AUG1 in an infected clone yielded mutant LoLV-K1, which was able to replicate in inoculated leaves of Nicotiana benthamiana, but not spread systemically. Mutation of AUG2 to UUG yielded mutant LoLV-K2, which was able to infect plants systemically. LoLV-K1 revertants that regained expression of a CP form of >28 kDa (by restoration of wild-type AUG1; by mutation to UUG at another site; or by mutation to an upstream UUG alternate initiation codon) were able to infect plants systemically. Substitution of four amino acids at the predicted cTP cleavage site combined with AUG2>CCC yielded mutant LoLV-C4, in which systemic infection was significantly delayed and symptoms altered. The N-terminal cTP sequence is crucial for efficient cell-to-cell and systemic movement, as well as homologous CP interactions and particle formation, but is not required for virus replication. Lack of mutation of the predicted cleavage site of the 28 kDa CP by either internal initiation or proteolytic cleavage limits systemic infection.

Aspergillus section Flavi populations in cornfields of Jalisco and its potential for aflatoxin contamination in maize

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Aflatoxin monitoring is the second key to dimensioning the contamination problem and suggest strategies to prevent the extent of contamination. The objectives of this research were to isolate toxigenic populations of Aspergillus section Flavi from soil and grain samples from seventeen municipalities of Jalisco, and determine the distribution of aflatoxin contamination. Some A. flavus and A. parasiticus populations were obtained from at least all municipalities of Jalisco. The highest frequency was recorded for A. flavus. The greater Aspergillus populations was associated to North Coast and South of Jalisco. For type of A. flavus strain, S-type (small sclerotia < 400 µm in diameter) population was only found in Puerto Vallarta. An important percentage (36%) of Aspergillus was described as unnamed taxon. In vitro not all isolates were capable to produce aspergic acid on AFPA medium.

Results with the rapid test of AflaCheck® by VICAM, shown only four differences in the response of leaves, stems and roots but showed both patterns in leaves. Leucine-rich repeat and DUF26 receptor-like kinases were the two receptor groups altered in all tissues. Genes encoding major intrinsic proteins, zinc and Fe(II) transporters were regulated in leaves and stems but unaffected in roots. Genes encoding ADP-glucose pyrophosphorylase, starch synthase, and starch branching enzyme were up-regulated in stems and leaves but repressed or unaffected in roots while amylase genes showed both patterns in leaves and stems. Sucrose transporter was up-regulated in leaves, sucrose synthase was repressed in stems but invertases showed both patterns in the two tissues. The implication of the differences in the response of leaves, stems, and roots to Las infection will be discussed to their distinct functions.

Detection of the begomovirus Clerodendrum golden mosaic China virus in Salvia splendens cv. Dancing Flame

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‘Dancing Flame’ is a popular salvia (Salvia splendens) cultivar with red flowers, described as having variegated foliage. After careful observations of plants at a local nursery in Baton Rouge, LA; we noticed that the variegated foliage resembled symptoms caused by plant viruses. The symptoms included bright yellow mosaic, yellow vein, and leaf distortion. Suspecting that a virus infection may be involved, we conducted graft and mechanical inoculations, dsRNA analyses and ELISA tests using antisera for several common plant viruses. From four healthy S. splendens cultivars reproduced the symptoms observed in ‘Dancing Flame’. ELISA testing and dsRNA analyses were negative. Suspecting an infection by a begomovirus, total DNA was extracted from a selected symptomatic ‘Dancing Flame’ plant and used as template for the rolling circle amplification (RCA) method. Two putative viral DNAs were obtained, cloned, and sequenced. Sequence comparisons of the 242 bp (ClGMCNV) and 273 kb (ClGDMCV) identified 98% identity with the corresponding genomic DNAs of Clerodendrum golden mosaic China virus (CIGMCNV) isolate YX1. The virus was detected by PCR using begomovirus-specific primers in all S. splendens plants showing variegated foliage but not in non-variegated plants. CIGMCNV was also detected in graft inoculated plants. These results strongly suggest that CIGMCNV is associated with the variegated foliage of ‘Dancing Flame’.

Does weed management for sweet corn differ with planting date?

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Sweet corn in the mid-Atlantic region of the U.S. is often planted over a two-month period to provide a consistent supply for either processing or fresh market. This requires a weed management program that is robust enough to control a wide range of weeds over a range of environmental conditions. A field trial was conducted in commercial sweet corn fields in 2009 and 2010 in Delaware on coastal plains soils. ‘Overland’ hybrid was planted over five planting dates, spaced 10-days apart. In 2009, planting began May 10 and ended June 20 and in 2010 dates were May 1 to June 10. Five weed control strategies were implemented, three soil-applied programs (s-metolachlor + atrazine [Bicep] and at reduced rates; full rate of s-metolachlor + atrazine + mesotrione [Lumax]) and two postemergence programs (s-metolachlor followed by carfentrazone + bentazon [Aim+Basgran]) and s-metolachlor followed by topramezone + atrazine [Impact]). Visual weed

trifloxystrobin significantly reduced disease starting 3 WAT; other fungicides reduced disease starting at 6 WAT. Timely applications of fungicides should reduce rust severity on field-grown gladiolus in Mexico, a leading source of cut gladiolus flowers, and, therefore, help reduce the movement of U. transversalis into the United States.

Comparative analysis of the host response of citrus leaf, stem and root tissues to infection by Candidatus Liberibacter asiaticus

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Candidatus Liberibacter asiaticus (Las) is known to cause citrus Huanglongbing (HLB) disease. Host response of leaf, stem and root tissues of Valencia sweet orange (Citrus sinensis) to Las infection was investigated using Affymetrix microarray. Using a 2 fold change and p < 0.05 as cut-off values, a total of 1008, 580 and 58 transcripts were up-regulated in leaves, stems and roots, respectively, while 1109, 350 and 58 were down-regulated, respectively. The following metabolic pathways were altered: transport, amino acid, hormone, lipid, secondary, carbohydrate, signaling, transcription and cell biogenesis. PR genes were mostly up-regulated in leaves and stems, and repressed in roots. JA genes were up-regulated in stems, down-regulated in roots but showed both patterns in leaves. Leucine-rich repeat and DUF26 receptor-like kinases were the two receptor groups altered in all tissues. Genes encoding major intrinsic proteins, zinc and Fe(II) transporters were regulated in leaves and stems but unaffected in roots. Genes encoding ADP-glucose pyrophosphorylase, starch synthase, and starch branching enzyme were up-regulated in stems and leaves but repressed or unaffected in roots while amylase genes showed both patterns in leaves and stems. Sucrose transporter was up-regulated in leaves, sucrose synthase was repressed in stems but invertases showed both patterns in the two tissues. The implication of the differences in the response of leaves, stems, and roots to Las infection will be discussed to their distinct functions.
control was collected by species. Smooth pigweed, common lambsquarters, common ragweed, and morningglory species were present both years and large crabgrass was present in 2009. Across all species, Impact was consistently the most effective treatment. Lumax was the most effective soil-applied program, but was not as consistent as Impact. For instance, morningglory control ranged from 58 to 82% for Lumax, while Impact ranged from 74 to 86% control. There were no trends that planting date influenced weed control or that weed emergence differed by planting date.

Did *Phytophthora ramorum* already invade Italian forests? A possible answer by mass sequence approach

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Phytopathology 101:S182

Since the first record of *P. ramorum* in nurseries stocks in Italy, Italian forests were largely inspected for *Phytophthora* by means of classical baiting methods followed by morphological and molecular identification. Results never reported the presence of *P. ramorum*. Recent introduction of mass sequence techniques in biodiversity studies provide the opportunity to analyse in one step, and high sensitivity, Phytophthoras population in forest soils. However outcomes of mass sequence must be treated with caution due to the risk of false positives, and need to be confirmed with species specific PCR and/or biological detection. Pyrosequencing analysis of beech and chestnut soils has been carried out in different sites in Italy to evaluate the diversity of Phytophthoras community. Sequence data analysed with dedicated database identified a range of Phytophthoras including species known to be common in forest soils in Italy. Some new species resulted to be present: among these, *P. ramorum* was not found in any of the soil samples taken in beech and chestnut soils. To confirm the detection, DNA’s utilised for pyrosequencing was amplified with species specific primers sets for *P. ramorum* and the amplicons sequenced. Sequences matched with *P. ramorum* on database. Next step will be to bait the soils in order to obtain *P. ramorum* living cultures. Cryptic presence of *P. ramorum* in forest would represents an improvement of knowledge on epidemiology and invasion mechanisms of this species in Mediterranean climate.

Testing bait sprays and male annihilation traps for area-wide management of the invasive fruit fly *Bactrocera invadens* in Senegal, West Africa

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Phytopathology 101:S182

*Bactrocera invadens* is a recently introduced invasive tephritid fruit fly. It has become the most economically important tephritid on mango since its recent arrival and expansion across Africa. A large-scale field trial was carried out in the main commercial mango production region of Senegal at a time when fruit fly pressure was high. Male annihilation using pheromone traps was compared to male annihilation plus weekly applications of a protein bait that attracts females. The performance of each treatment were replicated three times. To confirm the detection, DNA’s utilised for pyrosequencing was amplified with species specific primers sets for *P. ramorum* and the amplicons sequenced. Sequences matched with *P. ramorum* on database. Next step will be to bait the soils in order to obtain *P. ramorum* living cultures. Cryptic presence of *P. ramorum* in forest would represents an improvement of knowledge on epidemiology and invasion mechanisms of this species in Mediterranean climate.

Characterization of QoI resistant isolates in *Alternaria alternata* causing Alternaria brown spot in citrus

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Phytopathology 101:S182

Alternaria brown spot (ABS) is one of the most important foliar fungal diseases in tangerine hybrids. ABS affects leaves, twigs and young fruit, reducing yield and quality of the fruit. Q1 fungicides have been widely used for ABS control and have been the most effective products for ABS control registered for Florida citrus until recently. The site-specific mode of action of the Q1 fungicides, blockage of electron transfer in the mitochondria at the cytochrome bc1 complex, increases the risk of resistance. Therefore, resistance monitoring is essential for successful resistance management programs. Media amended with logarithmically-diluted fungicide and resazurin-based microtiter assays were optimized for determining the EC50 values for both methodologies. Isolates were more sensitive to pyraclostrobin than azoxystrobine. The EC50 values for sensitive isolates were less than 0.019 µg/mL and greater than 1.73 µg/mL for pyraclostrobin and resistant isolates. Values for azoxystrobine ranged from less than 0.607 µg/mL for sensitive to greater than 10 µg/mL for resistant isolates. Partial sequence of the cytochrome bc1 gene of resistant isolates revealed the expected GI43A point mutation as compared with the susceptible isolates. The presence of the point mutation correlated with the higher EC50 values of resistant isolates and explains observations of QoI fungicide failure for ABS in Florida citrus orchards.

Development of encapsulation methods for CO2 attractants and plant extracts as plant protection products

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Phytopathology 101:S182

Because of the demand for eco-friendly plant protection products there is a high interest in the development of formulation methods for natural substances such as plant extracts and attractants. The systematic development of formulation methods like encapsulation is essential to improve stabilisation, release and handling. Attractants based on CO2 have the potential to control the Western Corn Rootworm, *Diabrotica virgifera*. Because of the larvae’s orientation towards CO2-emitters CO2 beads can lure the larvae away from the roots. Artificial CO2 sources were successfully encapsulated in hydrogel beads. Experiments have shown a significant CO2 emission over two weeks. Furthermore, lipophilic CO2 plant extracts were encapsulated in hydrogel beads, and their antifungal potential was tested against the phytopathogenic fungi *Phytophthora infestans*, *Rhizoctonia solani AG1-IB* and *Phoma lingam*. The extracts were encapsulated in 2% Ca-alginate beads (2.8 mm diameter). For *Originum vulgare* (oregano) leaf extract (1.8 µg) and *Thymus vulgaris* (thyme) leaf extract (2.4 µL) agar diffusion tests showed considerable inhibitory effects. For *Allium sativum* (garlic) bulb extract (2.4 µg) only *P. lingam* was significantly inhibited. Further investigations will include the physico-chemical characterization of encapsulated plant extracts and the development of prolonged CO2-emitting capsules as well as attract and kill beads.

Current status of legume viruses in the Pacific Northwestern U.S.A.

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Phytopathology 101:S182

A survey was conducted to determine the prevalence of *Bean leafroll virus* (BLRV) on alfalfa and *Pea enation mosaic virus* (PEMV) on pea in commercial fields in Washington (WA) and Idaho (ID), U.S.A. Pea and alfalfa samples, randomly collected from commercial fields in Nez Perce (ID) and Whitman (WA) counties in June and August 2010, were tested for the presence of BLRV and PEMV by antigen-coated plate (ACP)-ELISA. Antiseria raised against the recombinant coat proteins (CP) of BLRV and PEMV were used. PEMV was found in one field out of 27 sampled in June, 2010 and in 20 out of the same 27 fields sampled in August, 2010. None of the samples tested positive for BLRV. PEMV consists of PEMV-1 (genus *Enamovirus*) and PEMV-2 (genus *Umbravirus*). The genomic sequences of the two species of PEMV-2 from selected samples obtained from this survey in ID and WA were characterized. Sequence comparisons and phylogenetic analysis of PEMV-1 and PEMV-2 sequences from ID and WA showed more than 95% sequence identity with isolates from Germany, UK and U.S.A.

Inverse responses of two major genes against bacterial blight of rice at different temperature regimes

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Transcriptome analysis using customized microarray platform was done to evaluate global gene expression patterns in *IRB24*, a bacterial blight resistant line carrying *xar* in comparison with the susceptible *IR24* at early stages of disease development. Differential gene expression including PR genes peaked
at 72 hrs post-inoculation in both genotypes, but bacterial population counts from whole leaves did not differ significantly. There were 152 differentially expressed genes (DEGs) between the resistant and susceptible genotypes. There were no differences in whole leaf counts but segment plating at the visible end of the blight lesions showed that bacterial counts across segments were not significantly different at warm temperature but significantly different among leaf segments at low temperature, suggesting host regulation of pathogen spread and symptom development but not pathogen multiplication. IRBB7, another resistant line carrying Sa7 shows increased effectiveness at high temperature regimes. Segment plating after the visible end of the blight lesions showed that bacterial counts across segments were not significantly different at 72 hrs post-inoculation in both genotypes, but bacterial population counts at low temperature, suggesting host regulation of pathogen spread and symptom development but not pathogen multiplication.

**Genome-enabled primer design to distinguish geographic origin of Xanthomonas oryzae from Taiwan and other places**

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*Yanthomonas oryzae* pathovars *oryzae* (Xoo) and *oryzicola* (Xoc) are the causal agents of bacterial leaf blight and bacterial leaf streak, respectively. While study of *X. oryzae* has focused predominantly on strains collected from Asia, diverse strains of *X. oryzae* have been isolated from infected rice in North and South America and Africa. Recently we used genomic sequence to design primers that differentiate *X. oryzae* strains by pathovar. In this study, we have analyzed draft genomic sequence of African Xoo and Xoc strains to develop specific diagnostic primers that distinguish Xoo and Xoc based on geographic origin. Primers were designed based on 40 predicted ORFs specific to 3 African Xoo strains, and 15 ORFs specific to African Xoc strains. The same strategy was used to design primers specific to Asian strains of Xoo and Xoc. Primers were validated on an extensive collection of DNA from *X. oryzae* and other bacteria collected worldwide. This study demonstrates how genomic data can be used to expedite design of tests that distinguish closely-related pathogens by geographical origin. These tests will serve as a valuable resource in the development of testing programs to rapidly identify and characterize *X. oryzae* in rice fields and seed lots. As sequencing costs continue to drop, genome-enabled ultraspecific diagnostics may become important epidemiological and regulatory tools for a variety of pathogens.

**Effect of the localization of Acidovorax citrulli in watermelon seeds on pathogen detection**

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Recently, we reported that in naturally infested watermelon seeds, *Acidovorax citrulli* localize under the seed coat, as well as within the pericarp or in endosperm tissues (when infected through the pistil of female flowers). However, the effect of the location of *A. citrulli* in seeds on pathogen detection has not been investigated. Hence, the goal of this study was to determine the effect of *A. citrulli* location in seeds on the ability to extract the pathogen by washing or crushing methods. Fifty infested watermelon seed lots were generated by inoculating female watermelon stigmas or by swallowing the pericarps of fruit ovaries with *A. citrulli* cell suspensions (~10^6 CFU mL^-1). Samples (~5 g of seed/lot) from pericarp and pistil-infested lots were crushed or washed for 60 minutes and genomic DNA was purified from the seed extract and subjected to real-time PCR. Samples (40-80 seeds/lot) from each lot were also tested by a modified seedling grow-out assay. For pericarp-infested lots, *A. citrulli* was detected in 86% of the seed wash samples as compared to none of the pericarp-infested lots. In contrast, when seed samples were crushed, *A. citrulli* was detected in 100% of the pericarp- and pistil-infested lots. BBF seedling transmission was observed for 100% of the seedlots from both infestation type, when planted under conditions of 28°C and 80% R.H. *A. citrulli* from seeds infected by pestil invasion, and that crushing is necessary for accurate pathogen detection.

**Nutritional cues and ambient pH modulate the in vitro activity of a polygalacturonase isozyme produced by Penicillium expansum**

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*Penicillium expansum* is an economically important pathogen of apple and pear fruit that causes blue mold in storage. This fungus produces polygalacturonase (PG) isozymes in decayed fruit which macerate host tissue and may be regulated by nutritional cues and ambient pH. Inhibition of this virulence factor along with understanding its underlying regulatory mechanism(s) could aid in disease control methods. Therefore, we investigated the roles of carbon and nitrogen nutrition and ambient pH, on *P. expansum* PG activity in vitro. The greatest PG activity was detected when the fungus was grown on a medium with apple pectin and ammonia as the sole carbon and nitrogen sources at pH 4. *P. expansum* PG activity was also affected by the form of galacturonic acid and the degree of pectin methylesterification when utilized as a sole carbon source. After 7 days of fungal growth in culture, the pH of the apple pectin-ammonia medium increased from 4 to 6, total soluble polyuronides decreased, and ammonia levels remained unchanged. A single PG isozyme with a pI of ~7.9 was produced in vitro which was different from those produced by *P. expansum* in decayed apple or pear fruit. Our results indicate that carbon, nitrogen, and pH modulate PG activity but do not affect the production of the single, prominent PG isozyme in culture.

**Suppressiveness to Phytophthora infestans infection in potato tubers by Alternanthera Mosaic Virus identified in clock vine in Florida**

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Clock vine (*Thunbergia laurifolia*) is a new host for *Alternanthera Mosaic Virus*. A sample of clock vine from a commercial greenhouse in Florida was cultured and showed virus-like symptoms including stunting, mosaic patterns, ringspots, and chlorosis. Approximately 10% of 50 plants were affected, including the mother plant used for vegetative propagation. ELISA tests using *Papaya Mosaic Potexvirus* antisera and microscopic observation of spindle-shaped inclusion bodies indicated the plant was infected with a potexvirus. Reverse transcription polymerase chain reaction (RT-PCR) using Miglino’s potex-4 and potex-5 primers produced an expected amplon of 280bp. The PCR product was sequenced in NCBI GenBank, resulting in a 97% identity match with *Alternanthera Mosaic Virus* (AltvMV) in GenBank accession DQ393785. AltvMV has been previously reported in Australia, Europe, North America and South America in ornamentals such as *Crossandra spp.*, *Phlox stolonifera*, *Portulaca grandiflora*, and *Scutellaria spp.*

**The role of silicon transport in improving plant disease resistance**

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Silicon (Si) is not considered as an essential element for plant growth yet its uptake is beneficial in alleviating abiotic and biotic stresses. These positive effects are variable since accumulation differs among plant species. This differential accumulation would be attributable to the presence of specific...
genes involved in Si uptake. These genes have first been recently described in rice with homologs reported in maize and barley. The objective of this project was to investigate, identify and characterize the presence of Si-transport genes in wheat, a species known to accumulate Si, and to determine their functionality and localization. Our results have allowed the identification and the cloning of a putative Si-transport gene presenting high homology (≥80%) with the Si-influx protein in rice known as Lsi1. Transient expressions of the wheat Lsi1 Si transporter (TaLsi1) coupled with GFP in Nicotiana benthamiana indicated that this protein was localized across the plasma membrane, a feature typical of other members of the Lsi1 family. The Si transport activity of TaLsi1 was confirmed in a heterologous system, Xenopus laevis oocytes, and its efficiency at transporting Si was comparable to that of the rice Lsi1. The discovery of these transporters provides a unique opportunity to understand and optimize the uptake of Si in a strategy to control plant diseases.

Incidence and prevalence of fungal pathogens on switchgrass seed produced in the U.S.A.

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Switchgrass (Panicum virgatum L.) production is increasing in acreage due to current initiatives for commercial biofuel production in the United States and worldwide. Due to a lack of seed certification programs for switchgrass, seedborne plant pathogens have likely been shipped along with the seeds to switchgrass producers. The aim of this study was to identify prevalent seedborne fungal pathogens of switchgrass from commercially available seed produced at the Southeastern cultivars, including ‘Alamo’, ‘Blackwell’, ‘Cave-in-Rock’, and ‘Kanlow’, from 12 sources were tested. A randomly-selected subsample of seed from each 454-g lot was surface-sterilized in 1% NaOCl for 1 min, rinsed three times with sterile water, and dried on sterile filter paper. Three hundred surface-sterilized seed per lot were plated on potato dextrose agar (PDA) amended with 100 mg/L chloramphenicol and incubated at 22°C. Seed were evaluated daily for development of fungal colonies. Emergent colonies were transferred to fresh PDA plates for identification. Rates of fungal infection among the 31 sampled seed lots ranged from 0 to 85.6%. The most prevalent pathogens isolated included Bipolaris sorokiniana, Bipolaris oryzae, Alternaria alternata, and Fusarium graminearum. Managing seedborne pathogens could increase stand establishment and crop yield, while decreasing the likelihood of a seedborne epidemic.

Wheat streak mosaic virus outbreak in North Dakota 2010

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North Dakota ranks second in the nation in total wheat production and is the largest producer of pasta wheat (durum), hard red spring wheat, and barley. Wheat streak mosaic (wsm) virus is a devastating disease of wheat and can also infect barley. In 2010, producers were concerned about this disease because of “a perfect storm” of conditions. Once the wsm virus is conveyed to producers with on-farm visits, newspaper, TV, and radio reports, producers are more likely to plant the next wheat crop. In response to calls from producers, consultants, and crop insurance adjusters, on-site visits were made to infected wheat fields. In addition to making visual assessments based on disease symptoms, ELISA testing confirmed the virus in 71 samples. As a result of the timely and effective response of NDSU extension, many of these producers were able to replant infected wheat fields with another non-host crop rather than suffer poor wheat yields and risk spread of the disease to adjacent fields. Best management practices to combat this disease were conveyed to producers with on-farm visits, newspaper, TV, and radio interviews, a user friendly management brochure, weekly updates in the NDSU Crop and Pest Report and numerous presentations.

Bacillus subtilis, strain QST 713: Soil applications for disease control, crop yield and quality enhancement

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Phytopathology 101:S184

Bacillus subtilis, QST 713, is a soil borne plant of plant growth promoting rhizobacteria. It is unique from other strains of B. subtilis in its production of anti-fungal and anti-bacterial products. These properties have previously been employed for the control of foliage plant pathogens under the trademark Serenade®. More recently, research has exhibited the advantages of soil applications of QST 713 in terms of disease suppression and beneficial plant effects leading to a product extension, Serenade® Soil. Soil applications, whether applied via seed treatment or drench, result in more vigorous plants as measured by topgrowth and rootmass. In the presence of soil borne pathogens QST 713 soil applications suppress disease with resultant increases in plant vigor, improved yields and, in some instances, quality. The aforementioned properties are the result of a protective biofilm on the roots of plants and disease suppression from an array of lipopeptides and other biochemicals known to suppress plant pathogens. Finally, plant modulating chemicals are also produced that may contribute to plant vigor and disease suppression. Practical implications of these new findings are discussed in regards to solanaceous crops, fruiting vegetables and cucurbits.

Two new broad-spectrum fungicides for use on pome fruits, stone fruits, fruiting vegetables and potatoes

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Merivon™ and Priaxon™ are two new broad-spectrum fungicides under development in the United States by BASF Corporation for control of key fungal diseases of pome fruits, stone fruits, fruiting vegetables and potatoes. Merivon is a premix fungicide containing two active ingredients, fluxapyroxad and pyraclostrobin in a 1:1 ratio and is currently being used for research on pome fruit and stone fruit diseases. Research in university and private cooperator trials has indicated Merivon is highly effective at controlling diseases such as scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha) of apple; blossom blight and brown rot of peach (Monilinia spp.) and powdery mildew of cherry (Podosphaera clandestina) in the rate range of 146 – 250 g a.i./ha. Priaxon is a premix fungicide containing a 2:1 ratio of pyraclostrobin to fluxapyroxad and is currently being used for research on fruiting vegetables and potato diseases. Research has indicated Priaxon is highly effective at controlling early blight (Alternaria solani) in both tomato and potato as well as powdery mildew (Leveillula taurica) and black mold (Alternaria alternata in tomato and black dot (Colletotrichum coccodes) in potato in the rate range of 146 – 300 g a.i./ha. Trial results from 2009 and 2010 will be presented. EPA registration is expected in 2012.

Grape hosts infested with glassy-winged sharpshooters produce volatile compounds which may attract egg parasitoids

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Glassy-winged sharpshooter (GWSS), Homalodisca vitripennis (Germar), is an important vector of Xylella fastidiosa Wells, which causes Pierce’s disease in grapes. Current management strategies in GWSS infested areas include mass release of the egg parasitoid Gonatocerus ashmeadi Girault and related species. However, little is known about egg parasitoid host finding behavior. Thus, volatile emissions from non-infested grapes and grapes infested with GWSS egg masses were compared using gas chromatography and mass spectrometry. Three compounds, tentatively identified as beta-ocimene, (1,8)-cineole, and beta-farnesene, were emitted in greater levels from grape hosts infested with GWSS egg masses than non-infested plants. Parasitoid attraction to synthetic versions of these compounds will be evaluated. If confirmed to be attractants for the egg parasitoids, these compounds could be deployed as liquid pheromones to attract parasitoids to local GWSS egg masses. If these compounds also could be screened in grape breeding programs aimed at reducing GWSS populations, because infested grapes producing more of these compounds will improve attractiveness to the parasitoids that prey on GWSS eggs.

Zebra chip disease is associated with increases in pathogenesis-related protein activity and host defense-associated secondary metabolites in tubers

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Zebra chip disease, putatively caused by the bacterium ‘Candidatus Liberibacter solanacearum’, is an emerging problem for potato growers throughout North America. However, little is known about the physiological changes that occur in diseased plants beyond the eponymous zebra chip symptom. One physiological change that occurs in diseased plants, presumably as a response to pathogen infection, is increased production of pathogenesis-related (PR) proteins. In this study we compared using liquid chromatography that diseased tubers, compared with non-diseased tubers, had twice the levels of beta-glucanase, exo-chitinase, and polyphenol oxidase;
and eight times the levels of peroxidase. Protein concentrations were also positively correlated with disease assessment ratings. Phenolic levels were much greater in diseased tubers, especially levels of chlorogenic acid derivatives and precursors. Zebra chip diseased tubers could exhibit increased browning when cut or fried because of these observed increases in enzyme levels and phenolic content.

*Xylella fastidiosa* infection of grapevines affects host secondary metabolite and defense-related protein levels within xylem

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Pierce’s disease of grapevine is a serious threat to grape production and is caused by the xylem-dwelling bacterial pathogen *Xylella fastidiosa*. Microscopy studies have documented morphological changes to grapevine xylem due to infection by *X. fastidiosa*. Comparatively, less is known about the biochemical interactions between *X. fastidiosa* and grapevine. In this study, phenolic content of xylem sap collected from non-inoculated and *X. fastidiosa*-inoculated grapevine was assessed using high-performance liquid chromatography. In addition, peroxidase, polyphenol oxidase, exo-chitinase, and beta-glucanase levels of non-inoculated and *X. fastidiosa*-inoculated grapevine were compared using enzymatic activity assays. Greater levels of four phenolics were observed in infected grapevine compared to uninfected grapevine. While beta-glucanase levels were reduced in infected grapevine compared to uninfected grapevine, effects of infection on other proteins was unclear. A better understanding of the role of phenolic compounds in grapevine defense against infection may aid in the development of novel management strategies. Furthermore, documented shifts in compound levels could be used to develop detection methods for Pierce’s disease that are host-based as opposed to pathogen-based.

Alteration of host gene silencing during root-knot nematode infection

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Root-knot nematodes (RKN, *Meloidogyne* spp.) are an agronomically important pathogen that are capable of establishing intimate feeding sites (giant cells) within the roots of a variety of plant hosts. Elucidating the mechanisms that allow RKN to establish and maintain these giant cells will be crucial in improving our ability to manage the economic damage they cause to crops worldwide. Much of the molecular underpinnings of this interaction however, remain unclear. We utilized functional genomic tools in order to gain more insight into what is necessary for a successful RKN infection. Trends observed in the transcriptome of laser-captured giant cells in *Arabidopsis thaliana* roots (microarray data obtained from 14 and 21 days post infection) suggest that the RKN infection process may be influencing mechanisms in host gene silencing. A subset of genes altered in their expression during nematode-infection are found to be up-regulated in Arabidopsis plants expressing the suppressor of gene silencing, He-Pro. Furthermore, genes normally down-regulated by trans-acting small RNAs are also up-regulated during the nematode infection process. Gene silencing is known to play an important part in plant defense responses to other pathogens, yet little is known with regards to how this pathway is involved during RKN infection. These results may help elucidate the role of gene silencing during the RKN infection process.

Biological control of invasive common ragweed, *Ambrosia artemisiifolia* L. with beneficial insect herbivores in China

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Common ragweed, *Ambrosia artemisiifolia* L. is unintentionally introduced into China in the 1930s. Since there are no effective biological control agents, it has rapidly spread to 21 provinces in China. Biological control of common ragweed has been conducted since the1980s in China, since several insect herbivores. *Epilobium streutmanae* and *Ophraella communa* were considered as two biological control agents of common ragweed in China. In recent decade years, many studies were focused on host specificity, biology, climatic adaptation, mass rearing and application of *E. streutmanae* and *O. communa*. The results indicated that both *E. streutmanae* and *O. communa* were safe and available. They reveal a higher fecundity under optimum temperatures, and are well adapted to subtropical climatic conditions. This suggests significant potential for using *E. streutmanae* and *O. communa* to suppress common ragweed because most of areas invaded by common ragweed belong to subtropics in China. Based on the biological and ecological studies, mass rearing of *E. streutmanae* and *O. communa* have been achieved in China. Since the spatial niches between *O. communa* and *E. streutmanae* are significantly heterogenous, combined control strategy of common ragweed with the two insect species were recommended and used in many areas invaded by common ragweed in China. Significantly, the two insect species can overwinter successfully, thus they can sustainable-suppress the population of common ragweed in the field.

Oviposition or host-feeding: Host handling strategy in the whitefly parasitoids *Eretmocerus hayati* and *Encarsia sophia*

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Host feeding provides nutrients that allow parasitoids to mature eggs. Thus, when handling an accepted host, the parasitoid chooses between current reproduction through oviposition and future reproduction through host feeding. Two whitefly parasitoid species, *Eretmocerus hayati* and *Encarsia sophia* were examined to determine if host density and host instar can affect their host handling decisions. In a single-instar no-choice experiment, the whitefly host, *Bemisia tabaci*, was introduced to *E. hayati* and *E. sophia* females at densities of 5, 10, 20, 30, 40, 50, 60, 70 and 80 second or third instar nymphs per 3.5 cm², respectively. Similarly, in a mixed-instar choice experiment, the whitefly host was introduced at densities of 20, 40, 60, 80 mixed-instar nymphs per 3.5 cm². It was found that with the increase of host density, more hosts were killed by females with host feeding and parasitism. The number of host parasitized by *E. hayati* peaked at the density of 40 nymphs per 3.5 cm². Meanwhile, there was a sharp increase in the number of host fed, indicating that the oviposition was prior to host feeding in *E. hayati*. A second mixed-instar choice experiment, the whitefly host was introduced at densities of 20, 40, 60, 80 mixed-instar nymphs per 3.5 cm². It was found that with the increase of host density, more hosts were killed by females with host feeding and parasitism. However, with the increase of host density, *E. hayati* was found to oviposit and feed both on the optimal host instars. In contrast, *E. sophia* females showed different host handling strategy that host-feeding was prior to oviposition.

Gut bacterial communities in the Bactrocera dorsalis and their luring activities on host

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In this paper, The 16S rDNA cloned libraries from the intestinal tract of lab-reared, lar steriliegur-reared and field-collected populations of B. dorsalis were compared. Phylogenetic analysis of 16S rDNA revealed that *Gammamatroclaus* was dominant in the all samples (73.0%–98.3%). Actinobacteria and Firmicutes were judged to be major components of a given library since they constituted 10% or more of the total clones of such library. The Flavobacteria, Deltaproteobacteria, Bacteroidetes, and Alphaproteobacteria were observed in small proportions in various libraries. LIBSHUFF analysis showed that the bacterial communities of B. dorsalis from the three populations were significantly different from each other (P = 0.008). Those results indicated that the intestinal tract of B. dorsalis adult contains a diverse bacterial community, and different environmental conditions and food supply could influence the diversity of the harbored bacterial communities and increase community variations. The whole beer, filtered and autoclaved supernatants of fermentation cultures of the cultured gut bacteria had attractiveness to *Bactrocera dorsalis*. However, autolaved supernatants were significantly more attractive than the whole beer or filtered supernatants. Six isolates, which autolaved supernatants were most attractive among all cultured bacteria, were selected to attract to B. dorsalis adults in field. Results showed that LRC38 was more attractive than others.

Food and microhabitat preferences of *Monochus*: A preliminary investigation

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Predatory nematodes are known to be potential nematode biocontrol agents, but they feed on nematodes opportunistically which means they also consume free-living nematodes and other microorganisms. Objectives of this project was to determine the efficacy of a commonly occurring predatory nematode, *Monochus*, to prey on plant-parasitic nematodes, and to explore microhabitats favorable for *Monochus* reproduction. DNA was extracted from individual *Monochus* isolated from fields infested with burrowing (*Radopholus similis*), or root-knot (*Meloidogyne* spp.) nematodes. To determine if *Monochus* habitually feed on plant-parasitic nematodes, PCR was conducted on the Mononchus DNA using primers specific for *R. similis* and *Meloidogyne*. The percentage of samples that tested PCR positive was evaluated to establish feeding preferences of Monochus in cultivated soils.
To examine favorable environments for Mononchus, 5 artificial microhabitats were inoculated with 6 Mononchus each. These microhabitats included: 1) 10 root-knot nematodes suspended in water at a 0.5 ml watch glass, 2) 10 g soil (frozen to free indigenous nematodes) inoculated with 40 root-knot nematode juveniles, 3) 3 g of vermicompost media, 4) water agar with a flamed carrot disc, co-cultured with Rhabditidae, and 5) 10 g soil amended with 1% (w/w) of dried sunn hemp (Crotalaria juncea) powder. Mononchus were extracted from these microhabitats 3 months after inoculation and counted.

**Genome-wide identification of genes regulated by ResB and ResC in Erwinia amylovora**

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The exopolysaccharide amylovoran is one of the major virulence factors in *Erwinia amylovora*, causative agent of fire blight of apples and pears. We have previously demonstrated that the ResB/BCD phosphorylase system is essential for virulence by controlling amylovoran biosynthesis. We have also found that the hybrid sensor kinase ResC differentially regulates amylovoran production in *in vitro* and *in vivo*. To further understand how the Res system affects *E. amylovora* virulence, we performed genome-wide microarray analyses to determine the regulons of ResB and ResC in liquid medium and on immature pear fruit. Our array analyses identified many novel genes differentially regulated by ResBC. Consistent with our previous findings, we confirmed that, when ResB/BCD acted as a positive regulator in both conditions, ResC positively controlled amylovoran biosynthetic gene expression *in vitro*, but negatively *in vivo*. Other virulence traits such as type III secretion, regulatory, and levensucrase genes were also regulated by the ResBCD binding sites in the intergenic regions of the *E. amylovora* genome. Predicted target genes were compared with ResBC-regulated genes identified in the microarray assay. Based on our findings, a working model has been proposed to elucidate how the Res phosphorylase system regulates virulence gene expression in *E. amylovora*.

**SSR markers closely linked with a major QTL on chromosome 12 associated with resistance to phytophthora strains of Ralstonia solanacearum in tomato**

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Phytopathology 101:S186

Bacterial wilt caused by phytophthora strains of *Ralstonia solanacearum* is a major disease in Southeast Asia. Tomato variety ‘Hawaii 7996’ has been shown to have durable resistance against the pathogen. Previous studies have associated the resistance with a major quantitative trait locus (QTL) on chromosome 12. This study constructed a new linkage map with good genome coverage. Simple sequence repeats developed from bacterial artificial chromosome sequences of tomato were the main marker types used. Attempts were made to rapidly develop resistance to *P. sojae* through different phenotypic methods and assessment of their contribution to yield

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Phytopathology 101:S186

Partial resistance to *Phytophthora sojae* in soybean is expressed as reduced infection efficiency, smaller root lesions and reduction in oospore production. This type of resistance is conferred by several quantitative trait loci (QTL). In several host-pathosystems, individual QTL have been reported to be effective towards specific pathogen isolates or influenced by environmental conditions. In addition, the contribution of QTL towards yield is an important factor for selecting QTL candidates for resistance breeding. In this study, QTL in the ‘Conrad x Sloan’ F₄ₛ population were mapped for three *P. sojae* isolates using two greenhouse phenotyping assays. Soybean QTL with smaller effects, especially those which originated from the susceptible parent, were not consistently detected among isolates or between phenotyping assays. Of the ten QTL mapped, four QTL on Chr. 18 and 19 from Conrad had the largest effect and were detected with all three isolates and both phenotyping assays. The RILs with resistant alleles from these four QTL had significantly higher yield than RILs with susceptible alleles. These results indicate the important role of these four QTL in conferring partial resistance to *P. sojae* populations as well as their contribution towards overall yield.

**Comparison of genes underlying two QTL conferring partial resistance to Phytophthora sojae from resistant and susceptible soybean genotypes**

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Phytopathology 101:S186
QTL on Chr. 19 in resistant cultivar Conrad were identified. Approximately 160 genes from these regions were amplified with long-range PCR, including 1.2 kb upstream and 400 bp downstream regions, from this resistant cultivar and the susceptible cultivar Sloan. Products ranged from 2-8 kb and were sequenced using Illumina GA. Reads were assembled against the sequenced soybean genome, Williams 82. A total of 1025 single nucleotide polymorphisms (SNPs) were identified from the amplicons between Conrad and Sloan. In comparison to both Sloan and Williams82, Conrad had 304 SNPs in 54 genes, and there were 11 genes in which Conrad sequence variation was unique. Twenty-nine SNPs were selected and verified by designing SNP markers using PCR Amplification of Multiple Specific Alleles (PAMSA) technique. This variation in sequence among these key genes may contribute to the difference in gene expression changes in expression levels in response to pathogen infection. Expression patterns of 20 genes in these regions in response to inoculation with *P. sojae* will also be discussed. This study provides additional SNP markers for fine mapping and marker-assisted resistance breeding for this trait.

Research on the elimination of CyMV and ORSV from *Phalaenopsis amabilis*

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The production and quality of orchid were influenced by Cymbidium mosaic virus (CyMV) and Odontoglossum ring spot virus (ORSV) in China. It was necessary to set up the technical system to get the virus-free orchid plant. *Phalaenopsis amabilis* was cultured in vitro firstly. Its formula of culture medium and culture procedure was optimized. The infected plants with CyMV and ORSV were tested by the bioassay and DAS-ELISA, and the tissue culture system of the infected plants was set up separately. The vigor plants were treated with the standard procedures of chemotherapy. The proliferation medium supplemented with a filter-sterilized solution of ribavirin 5, 10, 20, 50 mg/l respectively. 30 days later, Meristems (0.5-1.0 mm diameter) of plantlet were cut and cultured on shoot tip medium. Regenerated plants were tested by ELISA again. Frequencies of virus-free plantlets produced by ribavirin 20 mg/l treatment (85% for CyMV and 64% for ORSV) were higher than those by ribavirin 5 mg/l treatment (35% for CyMV and 0% for ORSV) and ribavirin 10 mg/l treatment (58% for CyMV and 0% for ORSV). Similar results were obtained with ribavirin 30 mg/l treatment (90% for CyMV and 71% for ORSV). Survival from ribavirin 20 mg/l treatment (83.6%) was higher than those from ribavirin 30 mg/l treatment (36.8%). When treated with 30 mg/l of ribavirin, the meristem became transparent and brown. The results above suggested that the chemotherapy of ribavirin was effective and could be used in the production of healthy orchid in future.

**Temperature effects on appressorial formation of Colletotrichum cereale**


Turfgrass anthracnose, caused by Colletotrichum cereale, is a devastating disease on annual bluegrass (AB) and creeping bentgrass (CRB). Intensified disease on annual bluegrass (AB) and creeping bentgrass (CRB). Intensified development was significantly hindered when compared to the other temperature treatments. These results suggested that the chemotherapy of ribavirin was effective and could be used in the production of healthy orchid in future.

**QTL analysis for transgressive resistance to root-knot nematode in a cotton RIL population derived from interspecific susceptible parents**


The root-knot nematode (RKN, *Meloidogyne incognita*) is a major parasite of cotton, causing significant yield losses in most production areas. A genetic standard recombinant inbred population of 138 lines developed from a cross between Upland cotton TM-1 (*Gossypium hirsutum*) and Sea island cotton Pima 3-79 (*G. barbadense* L.), both susceptible to RKN, was used to identify responses to RKN in two greenhouse experiments. Compared to both parents, 50.7% and 51.4% of lines showed less galling index and lower nematode egg production, respectively. Highly resistant lines were identified in the RIL population. Four quantitative trait loci (QTLs) accounting for 7.2 to 13% of the phenotypic variance (R²) in galling index, and two QTLs accounting for 6.7% and 8.4% egg production variance were identified based on interval mapping (LOD score ≥ 2) and Kruskal-Wallis (KW) analysis (P ≤ 0.005). These QTLs were located on chr3, 4 and 17 for galling index and chr14 and chr23 for egg production. In addition, 15 putative QTL accounting for 3.8% to 5.8% (2-LOD≥1 and P ≤ 0.05) of phenotypic variance in galling index, and 12 QTLs accounting for 3.2% to 5.2% in egg production were identified. In lines with combinations from both parents of 2 to 5 QTL with positive alleles, dramatic reductions of > 50% in both root galling and egg production were recorded. These epistatic effects in progeny derived from susceptible parents indicate that pyramiding these QTLs present a new level of nematode resistance in cotton.

**Nematode community analysis for soil ecosystem health prediction**

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Nematodes are good indicators of the structure and function of the soil ecosystem, and provide a reference for soil health conditions. However, performing nematode community analysis is laborious and technically challenging. The overall goal of this project is to develop a molecular tool that might replace conventional nematode community analysis. The first step of this approach is to identify four soil ecosystems in Hawaii with distinct soil health conditions, and to verify the reliability of conventional nematode community analysis. Four ecosystems examined including a forest site dominated by *Eucalyptus sp.*, an aboretum, a field, and a pineapple field. These indices would provide a standard to verify future molecular tools for nematode community analysis.

**Peg2, a novel pathogenicity gene in Magnaporthe oryzae encodes a transcription factor that activates and represses expression of distinct genes**

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**APSES proteins are a class of transcription regulators specific to fungi.**

In this study, PCG2 encoding an APSES transcription factor was isolated by T-DNA mutagenesis and was functionally characterized in *Magnaporthe oryzae*. The *pcg2* mutant shows defects in hyphal growth, conidiogenesis, and reduction in virulence. Peg2 is a protein localized in nuclei with two transcription activation domains, and could bind both the MCB-box and the SCB-box. Microarray analysis revealed that expression of 188 genes with either or both of the two boxes were activated or suppressed over two folds in the *pcg2* mutant, indicating that Peg2 is a transcription repressor and activator. Peg2 has two forms in mycelia, the full-length Peg2 and the truncated Peg2, and both were confirmed to function as a transcription activator and the truncated form as a transcription repressor. **MolTB1**, a novel gene with one MCB-box in its promoter region is activated in the *pcg2* mutant, and its constitutive expression resulted in slower hyphal growth and reduction in virulence. Our study is significant not only for identifying a novel pathogenicity gene but also for providing new insight into the mechanisms on how a protein functions as a transcription activator and repressor.

**Identification and characterization of Pectobacterium species causing potato blight disease in North China**

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Thirteen pectolytic strains were isolated from diseased plants, sampled from 7 different potato fields located in Inner-Mongolia, GanSu and HeBei provinces, and confirmed on CVP agar plate. By using the strain *P. carotovora* SCRI 1043 as positive control and based on the test of pathogenicity, ELISA, physiological and biochemical characteristic detection, 165 rDNA sequence analysis and specific PCR, it revealed that all strains were positive. Twelve of 13 strains were PLFA positive tested by PLFA specific primers. In conclusion, 12 strains can be identified as *Pectobacterium atroseptica*, and DL07 was considered as atypical *P. carotovora*. All of them were the pathogen and responsible for the occurrence of potato blackleg diseases in North China.

**Grosmannia clavigera**, a mountain pine beetle associated pathogen, has efficient ABC transporters for excreting monoterpenes or their derivatives. Y. WANG (1), S. DiGuistini (1), L. Lim (1), T. Wang (1), J. Bohlmann (1), C. Breuil (1)

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Phytopathology 101:S188

Western North American pine forests are being devastated by the mountain pine beetle and its associated fungi. **Grosmannia clavigera**, one of the most pathogenic fungi in this epidemic ecosystem, survives the toxic terpene/phenolic defense chemicals produced by host pines. Further, *G. clavigera* wild type grows with monoterpenes as a single carbon source, the mutant (GcABC1) cannot survive under these conditions, despite having other mechanisms to detoxify or utilize monoterpenes. We conclude that GcABC1 is a potential monoterpene efflux transporter that plays a critical role in removing toxic monoterpenes or their modified products from the fungal cell, allowing the fungus to grow in the presence of pine defense chemicals.

**Development of an in vitro bioassay to screen Prunus spp. for resistance to Armillaria ostoyae**

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Phytopathology 101:S188

Armillaria root rot is an important fungal disease of stone fruit trees within the genus Prunus. In Michigan, Armillaria ostoyae has been identified as the most prevalent species infecting tart cherry. There are no effective chemical controls available to the cherry industry, thus resistant rootstocks must be developed to reduce the decline and loss of tart cherry orchards due to Armillaria. An in vitro bioassay was developed to screen Prunus spp. for resistance to A. ostoyae. Seventeen Prunus spp. were evaluated for resistance to six strains of A. ostoyae. Branch segments (3cm) from two-year-old wood were planted adjacent to the leading edge of a 14 day-old A. ostoyae culture grown on yeast malt peptone glucose medium. The plates were incubated for 10 days at 25°C and then evaluated for penetration of the fungus into the host wood. The branch segments were cut longitudinally, and the periderm and cambium layers were peeled back to assess the extent and location of penetration by the fungal mycelial fans. The average range of mycelial fan penetration by the six A. ostoyae strains among the Prunus spp. was 9–18 mm, with the exception of P. maackii which exhibited an average penetration of 3.72 mm. Based on these results, P. maackii exhibited the highest level of resistance to A. ostoyae. Our data corroborate with previous research findings from in planta screening, and thus shows promise as a quick and reliable technique to screen Prunus spp. breeding stock.

**Construction of plasmid based expression vectors for the production of recombinant proteins in Xylella fastidiosa**

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Recombinant production of certain Xylella fastidiosa (Xf) proteins has proven difficult in commercially available *E. coli* and *P. pastoris* expression systems. Xf polygalacturonase (PG) is one of these proteins. Xf possesses a single PG gene and it was shown that if the gene encoding Xf PG was disrupted, the resulting PG-mutant was completely non-pathogenic in grapes. Thus, identifying peptides or proteins that could inhibit the activity of Xf PG may provide a viable means for protecting grapevines from Pierce’s Disease. To identify such PG inhibitors, it is necessary to produce adequate quantities of enzymatically active Xf PG. In order to express active Xf PG, we constructed a plasmid-based Xf protein expression system. These expression plasmids are based on the plasmid-based *Xf* protein expression system. These expression plasmids are modified products from the fungal cell, allowing the fungus to grow in the presence of pine defense chemicals.

**A common scab resistant potato cultivar is not explained by pathogen growth in soil or window of infectivity**

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Potato cultivars differ in resistance to common scab (CS); this might be due to differential growth of the pathogen at the plant root, or a shorter susceptibility window in resistant potatoes. Resistant (Superior) and susceptible (Chippewa) potato cultivars were inoculated by watering with a *Streptomyces* spore suspension at 10-fold differences in density over 5 orders of magnitude at a greenhouse. Tubers initiated 6 weeks after planting. CS severity was recorded on tubers 16 weeks after planting. The experiment was performed twice. Disease pressure was higher in the second experiment, but CS disease and *Streptomyces* growth patterns were similar. Quantitative PCR data from soil DNA extracted at 2 week intervals showed similar growth and timing of *Streptomyces* clinging to soil of both susceptible and resistant plants, while disease incidence and severity were very different. No clear window of susceptibility could be recognized in any cultivar, although disease was greatest when plants were initially treated at medium-high inoculum densities 2 to 4 weeks after planting. CS severity was lower at the highest inoculum density in both resistant and susceptible plants. The results showed that CS resistance was not explained through an inhibition of pathogen growth in soil on the root surface or by a shorter window of pathogen susceptibility in the resistant cultivar.

**House fly regurgitation spots may be a source of *E. coli* O157:H7 contamination of leafy greens**

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Phytopathology 101:S188

Fifth flies are known mechanical vectors of human enteric bacteria in hospital and restaurant settings. However, the role of flies in the movement of human pathogens to pre-harvest food plants is largely unknown. The fate of an attenuated strain of *E. coli* O157:H7 acquired by the house fly, *Musca domestica*, from contaminated manure and deposited on spinach via regurgitation spots was studied by molecular methods and scanning electron microscopy. Regurgitation spots were excited from spinach plants at 0, 4, 8, and 12 days after deposition by flies. Retention of bacteria on fly body parts was studied by relative quantitative PCR analysis of the *aeae* gene. It revealed that *E. coli* numbers in the regurgitation spots increased from day 1 to day 4, then dropped to levels comparable to the negative control spots. The same *E. coli* strain when acquired by flies from LB ampicillin plates did not increase in number, suggesting that manure-acquired *E. coli* O157:H7 was more capable of replication on the spinach surface than plate-acquired bacteria. Scanning electron micrographs of regurgitation spots from flies that acquired contaminated manure show bacteria-like organisms embedded in a dense matrix of regurgitated manure. These results suggest that fifth flies may pose a risk for contamination of leafy greens with *E. coli* O157:H7.

**Influence of genetic background of bacterial blight resistance gene Xa7 on population and movement of Xanthomonas oryzae pv. oryzae**

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Phytopathology 101:S188
Detection of sour skin of onion, caused by Burkholderia cepacia, using zNose technology

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Phytopathology 101:S189

In Georgia, sour skin of onion (Allium cepa), caused by Burkholderia cepacia, is responsible for the losses of onions in storage units that have a capacity of 40,000 bушels. Removing and re-grading onions can be an effective management strategy if the disease is detected early. zNose technology was explored as a new approach for the rapid detection of sour skin in stored onions. The zNose is a portable gas chromatograph used to rapidly identify volatile compounds in head space and can produce a volatile gas profile for the air in the storage room. Research was conducted in the spring of 2010 using Peruvian sweet onions and zNose technology to evaluate the differences between healthy and diseased onions. Surface disinfested onion bulbs were inoculated using a sterile toothpick contaminated with a 24 hour culture of B. cepacia which was inserted ~1cm into the shoulder of the onion bulb. Inoculated onions were incubated in sealed 2L glass jars for 72 hours, data were collected at 48 and 72 hours post inoculation. Also, onion bulbs infected with 10 different strains of B. cepacia were evaluated to determine if variation in volatile profiles was strain related. Results showed that the zNose can quantitatively differentiate between healthy onions and onions infected with B. cepacia after 3 days of incubation. There were no qualitative differences among volatile profiles produced by the 10 strains of B. cepacia.

A novel M RNA reassortant of Groundnut ringspot virus and Tomato chlorotic spot virus infecting vegetables in Florida


Phytopathology 101:S189

Groundnut ringspot virus (GRSV) was recently identified using serology and nucleocapsid gene sequence from tomato plants with severe tospovirus symptoms in South Florida, which extends the geographic range of this virus from South America and South Africa to now include North America. Full genome sequence analysis demonstrated that the Florida GRSV isolate was actually an M RNA reassortant with the S and L RNA segments coming from GRSV but with the M RNA segment coming from Tomato chlorotic spot virus (TCSV), a related but genetically distinct tospovirus species described in South America. This is the first report of a natural reassortant (i.e. L<sub>GRSV</sub>M<sub>TSV</sub>) between two tospovirus species. Regions of each of the three genomic RNA segments were sequenced to confirm that the L<sub>GRSV</sub>M<sub>TSV</sub> genotype was present in tomato samples collected in five south Florida counties starting in December 2009. The L<sub>GRSV</sub>M<sub>TSV</sub> genotype was also detected in pepper and tomato tospovirus symptomatic samples in December 2010. Western flower thrips (Frankliniella occidentalis) transmitted the L<sub>GRSV</sub>M<sub>TSV</sub> genotype and other thrips species are currently being investigated for their ability to transmit this virus. Neither parental genotype (GRSV or TCSV) nor alternate reassorted genotypes have been detected in any samples. These results suggest that L<sub>GRSV</sub>M<sub>TSV</sub> was introduced to the U.S. in its current form and that reassortment between distinct tospovirus species may be more frequent than previously thought.

Evaluation of leaf blight-resistant plant introductions of Brassica juncea and Brassica rapa and elucidation of inheritance of resistance

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Phytopathology 101:S189

Brassica leafy greens (Brassica juncea and Brassica rapa) represent one of the most economically important vegetable crop groups in the southeastern U.S. and worldwide. In the last 10 years, numerous occurrences of a leaf blight disease on these leafy vegetables have been reported in several states. One of the pathogens responsible for this blight is Pseudomonas cannabis pv. alisalensis (Pca). Two B. rapa (G30710 and G30499) and two B. juncea (P4148596 and G30988) plant introductions (PI) with moderate to high levels of resistance to this pathogen in greenhouse studies were tested for field disease severities and in comparison to eight commercial cultivars of B. rapa, B. juncea and B. oleracea, which include turnip greens, mustard greens, collard and kale. The two B. juncea PI and one of the B. rapa PI (G30499) were found to have significantly less disease than all tested cultivars except Southern Curled Giant mustard (B. juncea) and Blue Knight kale (B. oleracea). Inheritance of resistance studies performed with populations derived from the resistant G30988 and two susceptible, rapid-cycling PI indicate that the resistance is probably multigenic.

Making foliar fungicide applications to corn consistently profitable in Illinois

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Phytopathology 101:S189

Foliar-applied fungicide use on corn has increased recently in the North Central U.S. to manage corn ear rot and to reduce the number of fungicides to corn growers. In some cases, fungicides are being applied based on the potential of increasing yields without considering foliar disease risks or field scouting. To determine the effect of fungicides on corn yields in Illinois, trials were conducted at multiple sites across the state in 2008–2010. Products evaluated included quinoine outside inhibitor (Qol) fungicides, demethylation inhibitor (DMI) fungicides, and Qol-DMI mixtures. Fungicides were applied pre-emerge in comparison to eight commercial cultivars of B. rapa, B. juncea and B. oleracea (10 ear leaf area affected) approximately 21–30 days after application. In total, fungicides were evaluated in 21 different environments, which were divided into three different categories based on final disease severity levels on the non-treated controls (<10%, >10–15%, >15%). Fungicide applications were considered profitable if the yield response was at least 600 kg/ha. The mean yield response was 6, 419, and 949 kg/ha in low, medium, and high disease intensity environments, respectively. A yield response of at least 600 kg/ha was achieved 14%, 17%, and 75% of the time in low, medium, and high disease intensity environments, respectively. These results indicate that disease should be considered in order to achieve consistent profitable yield responses with foliar fungicides.

Identification of yeasts associated with grape sour rot in the north of China

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Phytopathology 101:S189

Sour rot becomes a very serious problem for grape growers in main grape production areas of China recently. It attacks grape berries and has a damaging effect on wine quality. The objective of this study was to identify yeast species associated with grape sour rot. The twenty-six strains of yeast were isolated by the tissue culture technique from the samples (diseased fruits and pulpae of Drosophila spp.), which were taken from four different vineyards in the north of China. To determine the pathogenicity of the isolated yeast strains, artificial inoculations were carried out in laboratory. Yeast identification was done by classical and molecular methods. On the basis of 26S rDNA D1/D2 domain sequence analysis, morphological and physiological characteristics, the yeast isolates were identified as follows: Pichia fluviatunm, P. membranaefaciens, P. novedgensis, Schizosaccharomyces pombe, Issatchenkia orientalis, I. terricola, I. scutulata, Arthroascus javanensis, Cyptococcus magnus and Hanseniaspora uvarum. Supported by the earmarked fund for Modern Agro-industry Technology Research System (mycrys-3t-bc-03).
An extensive survey on cereal cyst nematode (CCN) occurring in Jiangsu, Anhui, Shandong and Henan Province was carried out. The results indicated that CCN was detected in all 30 samples and the cyst density range was 1-80/100 g soil. The rDNA-ITS regions of the 30 CCN populations were amplified by PCR and the sequences were analyzed. The similarities of nucleotide sequences of 30 isolates are 97.7% to 100%. The phylogenetic tree of 30 CCN populations and other related species of Heterodera spp. reported in GenBank was constructed based on its rDNA-ITS sequences. The 30 CCN populations and the published CCN populations from China (AY148382, EU106175), Australia (AY148395) and Russia (AY148351) were clustered in the same group, showing high homology level in evolution. Characters of cereal cyst nematode (CCN) populations occurred on wheat in Jiangsu and other provinces were identified with molecular methods and might provide theoretical basis for control of the CNN disease.

**Development of PCR assay using simple sequence repeat primers for detection of 'Candidatus Liberibacter solanacearum'**

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Phytopathology 101:S190

Genetic variations of "Candidatus Liberibacter solanacearum" (Lso), the bacterium associated with the zebra complex of potato have been detected and differentiated based on simple sequence repeat (SSR) genotyping markers and sequencing of the amplicons of 16S-ISR-23S rRNA gene. More recently, 3 haplotypes have been reported with two present in North America, one in New Zealand and a third haplotype recovered from carrot in Finland. Current Lso detection assays are based on the 16S rRNA or 23S-ISR rRNA gene, since limited genetic variation exists in these regions among isolates, differentiating Lso strains has to be relied on sequencing the PCR amplicons which is expensive and time-consuming. In this study, a PCR assay was developed for both Lso strain detection and genotyping using one of our SSR primer pairs. The low detection limit of the PCR assay was approximately 100 copies of the target templates per reaction. This assay is more sensitive than previously published PCR assays using 16S, ISR or 23S rRNA gene and is able to distinguish two types of Lso by comparing PCR products on an agarose gel. This PCR assay has been validated using fresh or archived plant and psyllid samples associated with zebra complex disease obtained from potato commercial fields in the U.S.A. and Mexico. Both potato and psyllid samples were shown to have either type 1, type 2 or type 1 plus type 2 Lso.

**MCW-2 for management of root-knot nematode on carrot, tomato and cucurbits**

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Phytopathology 101:S190

Five RCB field trials with 5 replicates were conducted to evaluate the effectiveness of MCW-2 compared to an untreated control (UC) for the management of root-knot nematode (RKN), Meloidogyne javanica, on carrot, tomato, squash, cucumber, and cantaloupe. Treatments in all trials were MCW-2 at 2, 3, 4, 6, and 8 kg a.i./ha, oxamyl at 4.7 kg a.i./ha, metam sodium (MS) at 589 kg a.i./ha, 1,3-dichloropropane (1,3-D) at 84 kg a.i./ha and UC. 1,3-D was injected 14-days preplant. MS, MCW-2 and oxamyl were applied 7-days preplant followed by rototilling and sprinkler irrigation. Evaluations were conducted at harvest. The 3 and 8 kg rates of MCW-2 had a higher percent of marketable carrots. All MCW-2 rates and 1,3-D had fewer RKN. On tomatoes, 4 kg MCW-2 had a greater weight of fruit plus foliage. MS had a greater weight of fruit plus foliage and a greater weight of fruit. 3, 4, and 8 kg MCW-2 and 1,3-D had a lower root gall rating (RG). 4 and 8 kg MCW-2 and 1,3-D had fewer RKN. On cucumbers, 4 and 8 kg MCW-2 had a greater number, weight and size of fruit. MS and 4 kg had a lower RG. All treatments had fewer RKN. On squash, fruit size was greater for 4 and 8 kg MCW-2. MCW-2 at 2 and 4 kg had a lower RG. All treatments had fewer RKN. On cantaloupe, all treatments except 3 kg MCW-2 and oxamyl had a greater fruit weight. MCW-2 at 4 kg and MS had a larger number of fruit. MCW-2 at 3 kg had larger fruit. At 2 and 8 kg MCW-2 had a lower RG. All treatments had fewer RKN.

**Screening newly released Northwest Potato Variety Development Program cultivars for resistance to Pythium leak**

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Pythium leak caused by Pythium ultimum has been a major storage disease problem in the Pacific Northwest (PNW) potato production areas for many years, but in recent years reported losses have increased. In storage, Pythium-infected tubers rot quickly and create wet areas which enhances tuber breakdown, leading to severe losses. Current measures for controlling Pythium leak include fungicide applications of mefenoxam to foliage. Recently, mefenoxam resistant strains of P. ultimum have been found in PNW potato production areas. One potentially effective approach for controlling storage rots is through the use of resistant cultivars. In recent years, the Northwest Potato Variety Development Program (NPVDP) has developed new cultivars with higher disease resistance than the standards, e.g. Russet Burbank and Russet Norkotah. The objective of this study was to screen newly released NPVDP cultivars against Pythium leak to see if there are any differences in resistance to the disease compared to the standards. Tubers from each cultivar (Classic Russet, Clearwater Russet, Alpina Russet, Russet Norkotah, Premier Russet, Gein Russet, Alturas Russet, and Ranger Russet) were inoculated with P. ultimum by immersion in an inoculum suspension for 36 h. Tubers were then stored at 15°C for 2 weeks before being rated for disease incidence and severity. Results showed that several of the new cultivars had less than 5% disease in storage.

**Control of potato early blight tuber rot using post-harvest fungicide treatments**

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Phytopathology 101:S190

Early blight of potato caused by Alternaria solani is a common foliar disease found in most U.S. potato growing areas. Although primarily recognized as a foliar pathogen, A. solani can cause tuber lesions in certain cultivars. Tuber symptoms of early blight include circular to irregular lesions that are slightly sunken and often surrounded by a raised border. The underlying tissues are leathery to corky in texture and usually dark black with a yellow border. Lesions reduce the quality and marketability of fresh market tubers and present a challenge to potato processors as tuber lesions often require additional peeling to remove the darkened lesions and underlying tubers. The cultivar Western Russet is highly susceptible to both foliar and tuber early blight and growers have struggled to control the disease on tubers after placing potatoes in storage. The objective of this study was to screen a range of pre- and post-harvest fungicides for the control of tuber early blight. Naturally infected tubers were artificially bruised by tumbling and then treated with a range of fungicides before being placed in storage at 15°C for 3 months. Results showed that effective control of tuber early blight with registered post-harvest fungicides is possible. Tubers treated with phosphorous acid had significantly fewer lesions and smaller lesion diameters than the untreated control. These results and the results of the other products tested are presented and discussed.

**Potato virus Y resistance from Ry°cyd and Ry°sas genes: Practical application in a potato breeding program**

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Phytopathology 101:S190

In the Aberdeen Potato Breeding Program two different sets of clones and cultivars were tested for Potato Virus Y (PVY) R-gene markers. The first set of eighteen breeding clones/cultivars were selected based on Solanum tuberosum ssp. andigena, S. stoloniferum background, or known PVY resistance. R-genes in these species are reported to confer resistance against all PVY strains (extreme resistance). These clones/cultivars have been utilized in the breeding program as parents in a PVY resistance crossing block. A second set of clones derived from other parents with evidence of PVY resistance were selected based on acceptable agronomic characteristics. Both sets of were tested with SCAR marker RYSC3 for Ry°cyd and an SSR marker STM0003 for Ry°sas. All entries were further characterized by their PVY resistance on the field or by grafting in the greenhouse. Strategies to hybridize marker-confirmed PVY resistant parents with superior agronomic parents should result in higher frequencies of selected clones with extreme PVY resistance and desirable agronomic traits. Confirming PVY markers after initial agronomic selection would eliminate the need for greenhouse and field bioassays to determine resistance to individual PVY strains. Results show initial selection of ten russet types for processing or fresh pack and one for specialty. Of these eleven selections, three have markers for Ry°cyd and Ry°sas genes, while the remaining have a marker for either one of the genes.

**Variation in copy number, expression, and sequence of Avr1a/avr11 among populations of the oomycete plant pathogen Phytophthora sojae D. WICKRAMASINGHE (1), S. Stewart (2), A. Robertson (2), A. Dorrance (1) 

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Phytopathology 101:S190
Phytophthora sojae has reemerged as a prominent pathogen in some areas of the Midwest due to the pathogen’s ability to adapt to many of the resistant (Rps) genes, deployed in soybean cultivars. Recent research identified several Avr genes namely, AvrLa, AvrZh and Avr3c, and showed they belong to the RXLR family of effectors. Several mechanisms by which these effectors may contribute to changes in virulence in pathogen populations were proposed including the copy number variation of avirulence genes, differential regulation of the transcription of the genes and changes in amino acid composition of the proteins. However, this research only evaluated a few standard isolates. We compared the AvrLa locus across field isolates of P. sojae from Iowa and Ohio to discern which mechanism(s) maybe more critical at the population level. Preliminary data from both Iowa and Ohio suggest that the variation in copy number in the putative Avr3c gene may not be a major contributor to the variation in the avirulence/virulence response towards Rps1a.

Sporulation potential of Phytophthora kernoviae compared to P. syringae and P. cactorum on selected hosts

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Phytophthora kernoviae (Pk) is presently only found in the United Kingdom and New Zealand. There is concern that serious ecological damage could occur if it should enter the U.S. However, very little is known about the biology of this species. Oospore and sporangia production of Pk was compared on Rhododendron, Magnolia tripetata, Lirioidendron tulipifera, and Kalmia latifolia with two other species, P. syringae (Ps) and P. cactorum (Pc). Leaf disks of each host were inoculated with Pk, Ps, or Pc zoospores and set at 20 C in the dark. After 1 wk, the formed sporangia or oospores were counted. On Rhododendron and M. tripetata, Pk produced more than five times as many oospores as either Pk or Ps. On L. tulipifera, all species produced approximately the same amount, while on K. latifolia, Pk and Ps oospore production was almost nonexistent. Production of sporangia was different, resulting in significantly higher numbers for Pk on all hosts, except for Pc on Rhododendron, which was similar. These results show that secondary inoculum production of P. kernoviae via sporangia and oospores is more or similar to that of species already present in the U.S., and that the host plays a significant role.

Management of Phytophthora ramorum-infested nursery soil with Trichoderma asperellum

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Disinfection of Phytophthora ramorum-infested nursery sites is hindered by the inability to mitigate soil, a potential factor in recurrent infections. Current APHS-PPQ protocols for disinfection include steam sterilization, soil fumigation, and irrigation of contaminated or previously infested soil. However, there is a more feasible alternative for soil mitigation that is being investigated. Recent laboratory studies showed that Trichoderma asperellum (TA) can reduce soil populations of P. ramorum to non-detectable levels within 2 wk. To demonstrate the effectiveness of TA in a nursery setting, fall and spring field trials were carried out at the National Ornamentals Research Site at the University of California, Davis. The forty microplots were inserted into natural field soil in a raised bed and infested with P. ramorum chlamydospores 1 wk prior to treatments. Five treatments, including a non-treated control, a chemical, two commercially-available biological control products, and an experimental T. asperellum isolate (TA1), were applied separately to soil within a given plot in a randomized split-plot design. Soil samples were collected before and after treatment and monitored weekly for P. ramorum and Trichoderma spp. populations. After 2 wk, P. ramorum populations declined in all plots treated with TA1, while TA1 populations increased over time. These preliminary results suggest that T. asperellum is a promising alternative for managing P. ramorum populations in nursery soils.

Comparison of culture based and culture independent methods for identifying Rhizoctonia solani AG2-1 and 3 inhabiting infested plant material of potato

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Phytophthora 101:S191

Rhizoctonia solani causes stem and stolon canker on potato plants, as well as black scurf on potato tubers. AG2.1 is prevalent as mycelium and AG3 is less prevalent and survives as melanised sclerotia in potato field soil. Historically, AG3 is the most dominant anastomosis group isolated from potato plants but AG2.1 may not be easily isolated and therefore not implicated in causing stem and stolon symptoms. Cultivation-based analysis captures only isolates that are readily culturable or fast growing strains while cultivation-independent PCR based analysis allows the detection of non culturable or slow growing strains providing a more complete picture of the infecting strains. AG2.1 and AG3 were inoculated into soil in combination at various rates and one cv, Coliban minituber was planted per pot with 5 replicate pots, and grown in the glasshouse. Emergence was assessed 4 weeks after planting. Stolon pruning and stems canker lesions were assessed 4 times at 5, 8, 11 and 14 weeks after planting. Infected potato plant parts were divided into two with half being used for isolation of a culture and the other half being used directly for DNA isolation. qPCR analysis of cultures infected from tissue found that AG3 is the most common strain. Direct qPCR of infected plant material found that AG3 is the most common strain but the prevalence of AG2.1 increased using this technique. This research reveals that AG2.1 may be implicated in disease of potato.

The nature of the relationship between Soybean Cyst Nematode population densities and soil pH

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Phytophthora 101:S191

A consistent positive relationship exists between soil pH and soybean cyst nematode (SCN), Heterodera glycines, egg population densities in the field. The basis of this relationship is not understood. Soil pH may directly or indirectly affect SCN or the host status of soybean, and the relationship could involve other soil factors closely related to soil pH. To determine how quickly the relationship develops, greenhouse experiments were conducted using three different soils with low (5.6), medium (6.1), and high (7.5) pH. Soils were infested with 4,000 SCN eggs per 100 cm³ soil. After 30 days (time for one SCN generation), there were two and a half to five times more SCN females and eggs on a susceptible and a resistant soybean variety in the high pH soil than the medium and low pH soils, which were not significantly different. There were no differences in the numbers of eggs per female among the three soils or the two soybean varieties. Results were similar after 60 days. The results indicate that soil pH-SCN population density relationship develops quickly, which may imply that a direct effect is occurring. The experiment is currently being repeated and new experiments are underway in which SCN-infested soil with a pH of 5.5 was amended with three different rates of lime to increase the soil pH. SCN population densities and soil pH will be assessed at 30 and 60 days on resistant and susceptible soybeans grown in the soils receiving the different rates of lime.

Revisiting flag leaf-based foliar fungicide application thresholds for Stagonospora nodorum blotch management in soft red winter wheat

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Phytophthora 101:S191

Field trials were established in Illinois, Indiana, Ohio, and Wisconsin during the 2009/10 season to evaluate associations among disease severity at different crop growth stages and leaf positions and grain yield in an effort to establish fungicide decision thresholds. A randomized complete block design was used with two soft red winter wheat cultivars, one susceptible and one resistant to Stagonospora nodorum blotch (SNB), serving as whole plot. Plants were inoculated at either Feekes 6 or 9 (sub-plot) using four inoculum rates, 500,000 conidia/ml. SNB severity was assessed at weekly intervals at three different crop growth stages and leaf positions and grain yield in an effort to increase the soil pH. SCN population densities and soil pH will be assessed across all environments. Soil pH will be assessed at weekly intervals at three positions in the wheat canopy: on the flag leaf and the two leaves below the flag. In general, SNC increased over time and decreased from the lower to upper canopy. Based on preliminary results from linear mixed model covariance analyses, disease severity was greater in the middle of the canopy and at various growth stages had significant (P < 0.05) negative linear relationships with yield. Lower canopy disease severity had a positive linear relationship with upper canopy severity in many cases. This suggested that disease severity on lower leaves in the canopy could potentially be used to make early foliar fungicide application decisions to minimize the risk of severe damage to the flag leaf. Preliminary disease-yield models and fungicide decision thresholds based on these models will be discussed.

Efficacy of pre-flag leaf-based foliar fungicide application for Stagonospora nodorum blotch management in soft red winter wheat

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Phytophthora 101:S191

A consistent positive relationship exists between soil pH and soybean cyst nematode (SCN), Heterodera glycines, egg population densities in the field. The basis of this relationship is not understood. Soil pH may directly or indirectly affect SCN or the host status of soybean, and the relationship could involve other soil factors closely related to soil pH. To determine how quickly the relationship develops, greenhouse experiments were conducted using three different soils with low (5.6), medium (6.1), and high (7.5) pH. Soils were infested with 4,000 SCN eggs per 100 cm³ soil. After 30 days (time for one SCN generation), there were two and a half to five times more SCN females and eggs on a susceptible and a resistant soybean variety in the high pH soil than the medium and low pH soils, which were not significantly different. There were no differences in the numbers of eggs per female among the three soils or the two soybean varieties. Results were similar after 60 days. The results indicate that soil pH-SCN population density relationship develops quickly, which may imply that a direct effect is occurring. The experiment is currently being repeated and new experiments are underway in which SCN-infested soil with a pH of 5.5 was amended with three different rates of lime to increase the soil pH. SCN population densities and soil pH will be assessed at 30 and 60 days on resistant and susceptible soybeans grown in the soils receiving the different rates of lime.
The objective of this study was to evaluate claims that foliar fungicide applications prior to flag leaf emergence provide yield and economic benefits and adequate foliar disease control in soft red winter wheat. Field trials were established in Illinois, Indiana, Ohio and Wisconsin during the 2009/10 season. Two cultivars, one susceptible and one resistant to Stagonospora nodorum blotch (SNB), were planted in a randomized complete block design, with cultivar as whole-plot, fungicide treatment as sub-plot, and timing as sub-sub-plot. There were nine foliar fungicide treatments: separate full-rate applications of Headline and Prostaro at Feekes 5, 8, and 10; double half-rate applications of each fungicide at Feekes 5 and 8; and an untreated check. Cultivar was statistically significant (P < 0.05) for SNB severity in IL and OH and for yield in IL. Fungicide treatment had significant effects on SNB severity in IL, IN and WI, significant effects on yield in IL and OH, and marginal significant effect on yield in WI (P = 0.069). The effect of inoculation was not significant for SNB or yield at any location. Overall, the resistant cultivar had lower disease severity and higher yields than the susceptible cultivar. Fungicide applications at Feekes 8 or 10 resulted in lower SNB severity and greater yields than the check. In general, applications at Feekes 5 were not significantly different from the untreated check in terms of SNB severity and yield.

Effectiveness of early-season fungicide controls for the program of Sclerotinia homoeocarpa, the causal agent of dollar spot
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Dollar spot, caused by Sclerotinia homoeocarpa, is the most important turfgrass disease in the United States with respect to fungicide expenditures. Single early-season applications delay symptom development, but do not provide season long control of dollar spot. This field study compares the efficacy of a conventional dollar spot fungicide program to early-season programs. This study was conducted at the O.J. Noer Turfgrass Facility and at Milwaukee County Club in Wisconsin. Conventional applications started May 1, followed up with applications of a tank mixture of propiconazole and chlorothalonil. This program was compared to several early-season treatments applied May 1, followed up with applications of a tank mixture of propiconazole and chlorothalonil at either ¼ rates every 21 days or full label rates applied every 14 days. Treatments were arranged in a randomized complete block design with four replications with individual plots measuring 2.8 m². Disease severity was rated visually by counting individual dollar spot lesions. The effect of temperature on dollar spot development, but not to acceptable levels (<5% disease severity). The 28-day early-season program provided excellent suppression that was comparable to the conventional program. One fungicide application could be eliminated by using a 28-day early-season program instead of a 14-day conventional program, resulting in reduced expenditures and environmental inputs.

Effects of temperature on growth and aggressiveness of Sclerotinia homoeocarpa
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Dollar spot, caused by Sclerotinia homoeocarpa, is an important disease of most turfgrass species worldwide. S. homoeocarpa was described almost a century ago by F.T. Bennett, however the basic biology and epidemiology of the pathosystem is still unclear. Four isolates of S. homoeocarpa from Wisconsin and six isolates from Oklahoma were grown on native soil and USGA greens-grade sand. WI isolates were grown with and without creeping bentgrass (CRB) debris and incubated at temperatures ranging from 11 to 34°C. OK isolates were grown with CRB debris only at temperatures of 15, 20, 25, 30 and 35°C. Radial growth of mycelia was recorded at 24, 48, 72, and 96 hours post inoculation. Growth for all isolates was most rapid between 20 and 30°C. WI isolates grew best on native soil loam with CRB debris, while the effect of soil treatment was not significant for the OK isolates. Growth was limited and sporadic at temperatures below 15°C. To assess aggressiveness, CRB plants were inoculated with 3 WI isolates and 6 OK isolates and placed in separate growth chambers set at 10, 14, 20, 25, 30 or 34°C. Disease severity was visually assessed every 24 hours for four days. Disease developed in each temperature treatment, yet was most severe at 14 and 20°C for all isolates. These data indicate a temperature window for dollar spot development, but also demonstrate that another environmental parameter such as relative humidity is likely more important for dollar spot development.

Differential effects of host plants on accumulation, competition and transmission of curtoviruses from single and mixed infections
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Curly top disease, caused by viruses in the genus, Curtovirus, has impacted U.S. agriculture for over a century; however, over that period the viruses responsible for the disease have changed. The two most abundant curtovirus species today, Beet severe curly top virus (BSCTV) and Beet mild curly top virus (BMCTV) have not always been the dominant forms, and in some areas of the southwestern US new curtovirus species have been identified. To identify factors that drive the emergence of new species, as well as determine what factors cause a variant to become dominant, studies were undertaken to examine virus accumulation, competition and transmission among common weed and crop curtovirus hosts. Single and mixed infections of BSCTV and BMCTV were established in several weed and crop hosts, to determine efficiency of accumulation in each host plant species individually, as well as which virus dominates during mixed infections using TaqMan probes. Results indicated differential accumulation of each virus depending on host plant, and shifts in accumulation patterns during mixed infection. Transmission studies demonstrated variation in transmission efficiency of each virus among host plants. Evolution of the relationship between source plant virus concentration and transmission efficiency is ongoing. Results add to the knowledge of factors driving emergence and dominance among curtoviruses and contributes to overall knowledge of curtovirus ecology and epidemiology.

Endophytic colonization and induced resistance by Pseudomonas aeruginosa strain UPMP3
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Colonization patterns of oil palm (Elaeis guineensis) roots by a plant growth-promoting bacterium, Pseudomonas aeruginosa strain UPMP3, were studied in axenic conditions. UPMP3, both wild-type and tagged with gusA and gfp genes were used for enumeration and visualization of colonized root tissues of 3-month-old seedlings. Epiphytic and endophytic bacteria colonization was enumerated using dilution plating assays. Colonization patterns were visualized using fluorescent microscopy. The expression of two pathogenesis-related genes, chitinase and β-1,3 glucanase, in oil palm roots colonized with UPMP3 was monitored using RT-PCR for 28 days. There was significant increase in the rates of epiphytic and endophytic colonization by UPMP3. The bacteria entered the roots via joints at secondary adventitious roots adjacent to the elongation zone and first detected at the epidermal cells, then in cortex cells and finally at the surface of vascular tissues within seven days. Chitinase and β-1,3 glucanase were differentially expressed in oil palm roots where the highest expression for both genes occurred at 5 days after inoculation of the bacteria. These phenomena showed that the bacteria cells are capable of establishing themselves in oil palm roots and triggering some defense mechanisms of the host.
The use of natural plant volatile compounds for the control of potato blemish pathogens may be comparable to ear inoculations currently being used by the USDA. Seedling resistance to the fungus and bacterial growth were measured daily. Results are presented and discussed in the identification of six haplotypes. PCR-based analyses of two conserved TTSS (avrXoa1 and xopW) also differentiated the strains into distinct groups. avrXoa1 was detected in only 30% of African Xoc strains. Functionality of avrXoa1 was confirmed by leaf infiltration on rice Kitaake lines. Sequence analysis of xopW revealed three distinct groups among Asian and African Xoc strains, with African strains more diverse and rapidly evolving.

The use of natural plant volatile compounds for the control of potato blemish disease pathogens

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Phytopathology 101:S193

Many naturally occurring plant volatiles are known for their anti-fungal properties. However, they have limited use because they diffuse rapidly after coming in contact with air. In an initial study, acetohydroxy and 2E-hexenal were chosen as prototype volatiles in order to investigate the use of volatiles for control of blemish pathogens in fresh-pack potato packaging. Pure cultures of the three main potato blemish pathogens, Colletotrichum coccodes, Helminthosporium solani, and Pectobacterium atrosepticum were used in the study. Pathogen cultures were exposed to the pure volatiles in sealed jars for 7 days at 23°C. Results showed that 2E-hexenal was the more effective of the two volatiles with 2.5 μL providing complete inhibition of growth for all three pathogens. In the current study, experiments were repeated using inoculated tubers instead of pathogen cultures. The potatoes were inoculated by means of spore or bacterial suspension onto sterile filter paper that was placed onto a single or multiple wounds on the tuber surface. Pure volatile organic compounds were injected using a syringe into the headspace of sealed jars through an airtight valve. The headspace of the jars was sampled daily using SPME, and the volatile concentration measured by GC/MS. Fungal and bacterial growth were measured daily. Results are presented and discussed in relation to the future use of these volatile compounds in active packaging systems for the control of potato blemish diseases.

Influence of nickel on severity of pecan scab

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Phytopathology 101:S193

Pecan scab, caused by Fusarium effusum, is a major factor limiting profitability of pecan (Carya illinoensis) in humid environments. The effect of nickel (Ni) on the severity of pecan scab was examined in both field and lab studies in 2005 to 2010. Application of Ni sprays to foliage in tree canopies appeared to reduce subsequent scab severity. Host genotype influenced efficacy — those most resistant to scab (‘Desirable’) were most responsive to Ni treatment and those most susceptible (‘Wichita’ and ‘Apache’) were least responsive to Ni treatment. Addition of Ni to fungicide treatments delivered by air-blast sprayers to commercial orchards reduced the severity of scab on fruit by 6–52%, depending on the magnitude of the opposing fungicide. The severity of scab in the main trunk of selected trees was higher in Ni-treated orchards compared to controls.

Evaluating the use of solid-phase microextraction to detect aflatoxin-producing isolates of the fungus Aspergillus flavus

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Phytopathology 101:S193

Aflatoxins produced by Aspergillus flavus accumulate in maize during pre- and postharvest, and the detection of aflatoxin-producing isolates of the fungus is important to ensure food safety. To date, most methods used for detection are destructive. This study describes a potential non-destructive method utilizing volatile organic compounds (VOC’s). The objective of this project is to rapidly detect A. flavus in maize. Laboratory studies were initially conducted to examine the feasibility of utilizing this type of method. Headspace solid-phase microextraction (HS-SPME) and gas-chromatography/mass spectrometry (GC-MS) were used in these studies. Replicated experiments were performed in the laboratory employing an aflatoxin-producing isolate A. flavus, an atoxigenic isolate, or a media control for comparison. A volatile profile was assembled by GC-MS for all samples measured. A series of optimization experiments were performed including four types of SPME fibers (65-micrometer PDMS/DVB, 50/30-micrometer DVB/CAR/PDMS, 85-micrometer CAR/PDMS, and 85-micrometer PA) and five SPME fiber exposure times (30 minutes, 1 hour, 1.5 hours, 2.0 hours, 2.5 hours, 3.0 hours). Our data suggest that a 65-micrometer PDMS/DVB SPME fiber and a 30 minute exposure time was the optimum sample preparation technique. Future research will investigate the potential for examining and detecting VOC’s in whole plant systems infested with A. flavus in greenhouse-grown maize utilizing a portable Mini GC.

Evaluating resistance to Aspergillus flavus in maize genotypes using stem inoculations

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Phytopathology 101:S193

Few researchers have studied the systemic infection of Aspergillus flavus in maize. Our data suggest that fungal densities found within inoculated maize seedlings may be correlated with A. flavus kernel infection. These methods may be comparable to ear inoculations currently being used by the USDA. We also inoculated susceptible (S) and resistant (R) to A. flavus were inoculated by inserting a toothpick colonized with either an aflatoxin-producing isolate or an atoxigenic isolate into the stem. A sterile toothpick was used for comparison. Necrotic lesions observed in the S genotype inoculated with the toxigenic isolate were significantly larger than lesions observed in both R and S plants inoculated with the atoxigenic isolate and the control (P < 0.0001). Traditional isolations and quantitative polymerase chain reaction data detected the pathogen in tissue outside the visible stem lesion in both genotypes. A. flavus genomic DNA was detected 1.0 to 1.5 cm further from the edge of the necrotic region in the S genotype compared to 0.25 to 1.0 cm in the R genotype. These results indicate that genotypes resistant to kernel infection may have a mechanism that can limit fungal growth within the stem. Our data also suggest that the fungus is able to spread much farther within the stem tissues in susceptible genotypes compared to resistant genotypes.

Management of Sclerotinia blight of peanut in Texas: An integrated approach

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Phytopathology 101:S193

Sclerotinia blight, caused by the soilborne fungus Sclerotinia minor, is an important disease of peanut (Arachis hypogaea) in parts of Texas. The effects of fungicides, application timing and method, as well as partially resistant cultivars were evaluated from 2007–2010 in central and west Texas. Preventative applications of bosalid and fluazinam increased yields by 1814 and 1727 kg/ha, respectively over the non-treated control. Overall, preventative fungicide applications provided superior disease control and higher yields than applications targeted only for control of damping-off disease. Banded applications of fungicides improved yields by 147 kg/ha when compared to broadcast applications; however, differences in efficacy among application methods were more variable. Dramatic differences in disease incidence and yield have been observed between the cultivars evaluated. Use of the partially resistant cultivar, TamrunOL02 resulted in a 51% reduction in disease incidence and a 931 kg/ha increase in yield when compared to the commercial standard Flavorunner 458. Despite the increased level of resistance in Tamrun OL07, significant yield responses are observed when fungicides are applied.
Effect of temperature on potato psyllid reproduction and Liberibacter titer level in tubers

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Phytopathology 101:S194

“Candidates Liberibacter solancearum”, vectored by the potato psyllid (Bactericida cockerelli), is associated with zebra chip (ZC), a potato disease which persists in dark conditions rendering them unmarketable. As a newly emerging disease, little or no information is available on factors which influence ZC epidemiology. To determine the effects of temperature on psyllid reproduction and Liberibacter titer, growth chamber studies were conducted at 4 constant temperature levels (15, 21, 27, and 32°C). Potatoes were grown in caged pots (4 pots per cage) and after two weeks, plants were similarly set up in uninfested cages to serve as controls. Development of new psyllids was monitored periodically using yellow sticky traps. At the end of the test, tubers were harvested and tested for Liberibacter titer levels using qPCR. Development of new psyllids was delayed by 10 days in the 21°C chamber and the number of new adults was substantially lower compared to those in the 27°C chamber. No new adults were observed in the 15C and 32°C chambers. Tubers from all psyllid-infested plants tested positive for Liberibacter but tubers from the 15C chamber had the lowest titer compared to those from the other temperature chambers. The results suggest that cool temperatures slow psyllid reproduction and the buildup of Liberibacter titer in tubers. This finding represents a significant addition to our understanding of the epidemiology of ZC in relation to temperature.

Fungi in Botryosphaeriaceae causing stem blight in the Southeast and latent infection in southern highbush blueberry propagative material

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Stem blight of southern highbush blueberries (SHB) in Florida is caused by a species complex in Botryosphaeriaceae (Bot) that includes Botryosphaeria dothidea, Lasiodiplodia theobromae, and Neoscytascicum ribis. In 2010, 365 stem blight samples were collected from SHB and rabbiteye cultivars from 28 sites in the southeastern United States (AL, FL, GA, NC, and SC); 86% of the samples were initially identified as Bot species. Sequence analysis of fungal ribosomal DNA (rDNA) was used for phylogenetics and to design PCR restriction fragment length polymorphism (PCR-RFLP) assays to discriminate among Bot species. Neoscytosccicum ribis and L. theobromae were identified as the two predominate species causing stem blight in the southeastern U.S.; Botryosphaeria corticis, B. dothidea, and Diplodia seriata were found infrequently. In an additional survey, propagative material was collected monthly from May to October and fungal isolations identified latent Bot pathogens on apparently healthy softwood cuttings (swc). Bot fungicides were used from swc. Using PCR-RFLP, L. theobromae and N. ribis predominantly identified from both isolations; pathogenicity of select isolates was confirmed. Our results suggest up to 45% of swc could have latent infections. Additional research is needed to determine the impact of these latent infections on blueberry production and pathogen distribution.

Evaluate Actigard applied through drip irrigation for suppression of Xanthomonas contamination in carrot seed

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Bacterial blight caused by Xanthomonas solani pv. carotae (Xhc), is the most important disease on carrot seed crops in the Pacific Northwest because the primary inoculum for this disease in commercial carrot production. Currently, there is no effective way to control seed contamination, and seed lots commonly require hot water-treatment, which is expensive and reduces seed vigor. The objective of this study was to evaluate Actigard, a systemic resistance inducer, applied through drip tapes for suppression of X. carotae, and inoculated with Xhc seed crops. In two greenhouse experiments, 6-week-old carrot seedlings in 4-inch pots were drenched with 0, 5, 10, and 15 mg Actigard, and inoculated with Xhc suspension 1, 3, and 6 wk after. The results show that Actigard drenches were inconsistent. Suppression of Xhc was observed positively related to the Actigard dose on plants inoculated 6 wk post-drench in the first experiment and 3 wk post-drench in the second experiment. In a field trial, Actigard applied through drip tapes twice to three times at 2 to 8 oz/a was compared with the commercial standard, two foliar ManKocide sprays. Similar to ManKocide, Actigard applied through drip irrigation reduced Xhc contamination on carrot seed although it did not control Xhc population on leaves and umbels. The results revealed that Actigard through drip is promising for suppression of Xhc on carrot seed, and more studies are needed to optimize method, timing and dose of Actigard application.

Effect of vulenic acid produced by Nymbia alternantherae on chloroplast function of alligatorweed

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PEOPLES REP OF CHINA

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Vulenic acid produced by Nymbia alternantherae can cause leaf blight of alligatorweed. We investigated the effects of the toxin on chloroplast function of alligatorweed and its mode of action. Thylakoids were isolated and treated with the toxin at concentrations of 0.042, 0.083, 0.208, 0.292, and 0.417 mMol/L. Thylakoid suspensions were then analyzed for electron transport rates of the whole chain, of photosystem II (PS II) and of photosystem I (PS I), and for the activities of non-cyclic and cyclic photophosphorylation, and of Mg2+-ATPase and Ca2+-ATPase. Phosphate buffer at pH 4.1 and double distilled water were used as controls, with three replicates for each treatment. When treated at a concentration of 0.417 mMol/L, the electron transport rates of PS II and the whole chain, the activities of non-cyclic photophosphorylation and Mg2+-ATPase and Ca2+-ATPase were reduced by 48.0%, 60.6%, 42.0%, 41.0% and 39.1%, respectively, compared to the phosphate buffer-treated control. The electron transport rate of PS I and the activity of cyclic photophosphorylation decreased by 16.0% and 7.5%, respectively, at the same concentration. Phosphate buffer content increased little after the detached leaves were treated for 48 h at 0.417 mMol/L. These results suggested that the toxin might affect the chloroplast function of alligatorweed, and its main action might be the inhibition of electron transport in PS II and of the activity of non-cyclic photophosphorylation.

Susceptibility to nucleopolyhedrovirus and mitochondrial DNA sequence variation among different geographic populations of Ectropis oblique Plout

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PEOPLES REP OF CHINA

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The tea looper, Ectropis oblique Plout, is a recurring major tea pest that is widely distributed in South China. Ectropis oblique nucleopolyhedrovirus (EoNPV), as a commercial baculovirus insecticide, has highly toxicity to tea looper larvae. In recent years, some field observations suggested that EoNPV was failing to control tea production damage. The laboratory bioassay experiment was indicated that was a significant difference of susceptibility to EoNPV among the different geographic populations. Compared with the susceptible population, EoNPV virulence ratio of the resistant population was substantially lower compared to those in the 27°C chamber. No new adults emerged at the same time. Photophosphorylation rate and the electron transport rate were reduced by 48.0%, 60.6%, 42.0%, 41.0% and 39.1%, respectively, compared to the phosphate buffer-treated control. The electron transport rate of PS I and the activity of cyclic photophosphorylation decreased by 16.0% and 7.5%, respectively, at the same concentration. Phosphate buffer content increased little after the detached leaves were treated for 48 h at 0.417 mMol/L. These results suggested that the toxin might affect the chloroplast function of alligatorweed, and its main action might be the inhibition of electron transport in PS II and of the activity of non-cyclic photophosphorylation.

Dissect the evolutionary process of Potato virus Y to overcome host resistance during single-host passages

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Resistance breaking (RB) variants of Potato virus Y (PVY) can emerge during single infection events of the avirulent PVY-NN in partially resistant tobacco varieties. Both tobacco lines contain the recessive resistance gene N-J tree showed that 36 unique haplotypes diverged into 2 groups which have 5% of genetic difference. Correlated to the result of susceptibility bioassay, the group one was more susceptible with EoNPV infection than the group two.

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The deduced polyprotein sequence contains conserved motifs found in amino acid residues. Its genomic organization is typical of potyviruses, and comprises a large open reading frame encoding a single polyprotein of 3066 amino acid residues. The polyprotein, designated VPg (viral genomic), is covalently linked to the 5′ end of the viral RNA and contains motifs that are conserved among most potyviruses.

Presence of Gooseberry vein banding associated virus (GBVAV), a badnavirus in the family Caulimoviridae, is strongly correlated with gooseberry vein browning disease in Ribes spp. In this study, full-length genomic sequences of four GBVAV isolates from different hosts and geographic regions were determined to be 7649-7663 nucleotides. These isolates share identities of 96.4-97.5% for the complete genomic sequence, indicating low genetic diversity among them. The GBVAV genome contains three open reading frames (ORFs) on the plus-strand that potentially encode proteins of 26, 16 and 216 kDa. The size and organization of GBVAV ORFS 1-3 are similar to those of most other badnaviruses. The putative amino acid sequence of GBVAV ORF 3 contained motifs that are conserved among badnavirus proteins including aspartic protease, reverse transcriptase and ribonuclease H. The highly conserved putative plant RNA-dependent binding site is also present in the 935-bp intergenic region of GBVAV. The identities of the genomic sequences of GBVAV and other badnaviruses range from 49.1% (Sugarcane bacilliform Mor virus) to 51.7% (Pelargonium vein browning virus, PVBV). Phylogenetic analysis using the amino acid sequence of the ORF 3 putative protein shows that GBVAV groups most closely to Dioscorea bacilliform virus and Taro bacilliform virus. These results confirm that GBVAV is a pararetrovirus of the genus Badnavirus.

**Biological characterization and complete genomic sequence of Carrot thin leaf virus**


A cilantro isolate of Carrot thin leaf virus (CTLV) from diseased plants in a commercial field in California was characterized. The experimental host range of the virus included 15 plant species in the families Apiaceae, Chenopodiaceae and Solanaceae. Almost all infected plant species showed symptoms of local lesions, chlorosis, stunting and/or thin leaf. CTLV was transmitted to all 9 host species in the Apiaceae by green peach aphids. It reacted with the potyvirus group antibody in both ELISA and western blot analysis. The complete genomic sequence of CTLV was determined to be 9,491 nucleotides, excluding the 3′ poly(A) tail. The CTLV genome comprises a large open reading frame encoding a single polyprotein of 3066 amino acid residues. Its genomic organization is typical of potyviruses, and the deduced polyprotein sequence contains conserved motifs found in members of the genus Potyvirus. Comparisons with available genomic sequences of other potyviruses indicate that CTLV shares 22.4-56.4% identities with species of the existing genera and unassigned members in the family Potyviridae at the polyprotein sequence level. Phylogenetic analysis based on the deduced sequences of the polyprotein and individual proteins indicates that CTLV is distinct from Carrot virus Y (CarVV) and several CarVV-related potyviruses infecting apiaceous plants.

**Radiosynthesis of tritium-labelled and the stability of novel cis-configuration nitromethylene neonicotinoids**

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Recently, novel cis-configuration nitromethylene neonicotinoids were developed as insecticides in China, such as Paichongding and Cycloalexid. The metabolism and stability of these two compounds are our focus. The radiosynthesis of [3H2]-Paichongding and [3H2]-Cycloalexid were achieved using NaB3H4 reduction. The labelled compounds could be used as radiotracers for further study of metabolism and toxicology. In addition, the photodegradation of cyanomethylene compounds with cis-nitro configuration had been studied in distilled water. The degradation pathways of Paichongding were proposed according to the structures of photolytic products.

**Colonization of tomato seedlings by bioluminescent Clavibacter michiganensis subsp. michiganensis under different humidity regimes**


The dissemination of Clavibacter michiganensis subsp. michiganensis (Cmm), the causal agent of tomato bacterial canker, is facilitated by mechanical wounds that are easily made during seedling production and crop maintenance. Little is known regarding translocation of Cmm in tomato seedlings through wound infection or the influence of environmental factors on Cmm growth as an endophyte. A virulent, stable, constitutively bioluminescent plant Cmm strain BL-Cmm 17 coupled with an in vivo imaging system (IVIS, Xenogen), a quantitative low-light device, allowed visualization of the Cmm colonization process in tomato seedlings in real-time. The dynamics of bacterial infection in seedlings through wounds were compared under low (45%) and high (83%) relative humidity. Bacteria multiplied rapidly in coryledon petioles remaining after clip inoculation and moved in the stem towards both root and shoot. Luminescent signals were also observed in tomato seedling roots over time and root development was reduced in inoculated plants maintained under both humidity regimes. Wilting symptom development was more severe in seedlings under high humidity regimes. A strong positive correlation between light intensity with bacterial population in planta suggests that bioluminescent Cmm strains will be useful in evaluating the efficacy of bactericides and host resistance.

Identification of an RNA silencing suppressor encoded by Southern rice black-streaked dwarf virus S6

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Southern rice black-streaked dwarf virus (SRBSDV), a member of the genus Fijivirus within the family Reoviridae, is a novel virus that has been observed on rice (Oryza sativa) in Guangdong Province and Hainan Province recent years. In this study, we identified an RNA silencing suppressor encoded by SRBSDV. SP6, encoded by SRBSDV segment 6, exhibited silencing suppressor activity in co-infiltration assays with the reporter green fluorescent protein (GFP) in transgenic Nicotiana benthamiana line 16c carrying GFP. It interfered with systemic and silencing induced by sense RNA, but did not interfere with local and systemic silencing induced by dsRNA. SP6 can reverse the GFP silencing as well as prevent long distance spread of silencing signals which have been reported to be necessary for inducing systemic silencing in host plants. Expression of SP6 enhanced Potato virus X pathogenicity in N. benthamiana. Collectively, our results establish SP6 as a suppressor of RNA silencing encoded by a plant dsRNA virus and further suggest that SP6 targets an upstream step of dsRNA formation in the RNA silencing pathway.

**Plant novel activator PRDA-003 for soil-borne disease**

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The plant disease-resistant activator is one kind of new plant protection variety with the green-chemistry concept. Plant scientists play attention to it because of its potential comprehensive superiority to other kinds of agricultural protecting agents. The plant activators don’t have notable antimicrobial activities in vitro, but they can induce their immune system in vivo to protect themselves from being invaded by plant diseases. Based on BTH, we have designed and synthesized a series of new BTH analogues. Their biological activities were also studied. At first, we studied that the novel synthesized plant cell culture elicitors induce SAR activities on pathogen in vitro. The results showed these elicitors didn’t have directive pathogens inhibition, which corresponds to the properties of plant activators. Then we studied the activators inhibiting Soil-borne disease. PRDA-003 has excellent inhibition on Cylamen root rot disease, up to 92.3% inhibition in 25 mg/L. Also, the disease-resistant has sustainability, up to 35-day. PRDA-003 also has disease-resistant on Potato scab 58.88% in 250 mg/L.

**Concentration and cultivar effects on efficacy of ACM941-CL01 biofungicide in controlling Fusarium head blight of wheat**

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Fusarium head blight (FHB) is a destructive disease of wheat. This research was to examine the effect of concentration and cultivar on the efficacy of ACM941-CL01, a formulated product of Clonostachys rosea strain ACM941, in controlling FHB and deoxynivalenol (DON) contamination in wheat. Seven concentrations of ACM941-CL01, ranging from 104 to 108 CFU/mL, were tested for the control of FHB and significant effects observed for concentrations at or above 8×10^5 CFU/mL in the greenhouse trials or 3×10^6
late onset of symptom development in the production system. Seed producers rely on seed assays as the final assurance of producing pathogen-free seeds making test reliability and sensitivity paramount. The current National Seed Health System (NSHS) accredited tests for BFB are either slow and expensive and/or require the use of multiple extremely toxic reagents. A safer, faster, real-time PCR method was first reported in 2009 and here we report on experiments comparing the method with other NSHS accredited methods. Using naturally infested seed lots, artificially infested seeds, and low level Aac spiked seed wash solutions, significant differences in detection were found among the methods. The Seminis Inc. PCR-Wash, and Syngenta SYBR Green methods detected Aac more frequently than the Seedling Grow-out (p = 0.027). The Aac spiked seed wash solutions revealed the Syngenta SYBR Green method was more sensitive at low infestation levels than the Seminis Inc. PCR-Wash method (p < 0.01). The DNA analysis of extractions from different curcubit lots is reported.

**Genome-wide identification of virulence factors of citrus canker pathogen Xanthomonas citri ssp. citri using a transposon mutagenesis strategy**

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Phytopathology 101:S196

Bacterial citrus canker disease, which is caused by Xanthomonas citri ssp. citri, is one of the most devastating diseases on citrus. To investigate the virulence mechanism of this pathogen, a X. citri ssp. citri 306 mutant library was constructed by randomly mutagenesis using EZ-Tn5. Around 22,000 independent mutants were inoculated and screened in host plant citrus individually. And 216 mutants were identified which showed significant virulence reduction. Southern blotting assay revealed only one mutant contains two EZ-Tn5 insertions. To determine the insertion site of transposon, a rescue cloning method was used to clone the flanking sequences of EZ-Tn5. 102 genes/loci were identified. Among them, 99 genes/loci were mapped on the chromosome; three and one genes/loci were mapped on the magaplasmid pXAC64 and pXAC33, respectively. Interestingly, 11 genes/loci were overlapped with the HrgG regulon which has been reported recently by our group. In addition to the known virulence factors such as type II, III and IV secretion systems, cell-cell signaling system and EPS, LPS biosynthesis systems, many novel genes were uncovered in this work. For example, three transcriptional regulators and 16 hypothetical genes were identified and their roles in virulence of Xanthomonas have not been determined previously.

**Development of a species-specific PCR assay to identify the cereal cyst nematode Heterodera filipjevi**

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Heterodera filipjevi is one of the most economically important cereal cyst nematode species that restrict the movement of crops around the world. It is found in winter wheat fields in Oregon, U.S.A. Accurate identification of cyst nematode species in affected fields is essential for providing effective management strategies. It is difficult and time-consuming to distinguish H. filipjevi from other closely related Heterodera species based on morphological characters. A species-specific PCR assay was developed to detect and identify H. filipjevi. H. filipjevi was found in wheat from eight different cropping systems. A primer set was designed from the internal transcribed spacer (ITS) region of Heterodera rDNA. This primer pair was highly specific when tested on four other Heterodera species, seven non-Heterodera plant-parasitic and non-parasitic nematodes, and six fungal pathogens associated with wheat root diseases. This primer pair was also predicted to be specific by in silico analysis using the ITS regions of 35 other accessions of 14 Heterodera species and 26 accessions representing other 17 nematode species. Optimized PCR conditions were established and H. filipjevi was detected and identified by a specific PCR fragment of 170 bp. This PCR assay was rapid and reliable, and was able to detect single eggs and juveniles of H. filipjevi among all tested nematode species. The species-specific primers are currently being tested for use in a real-time PCR assay, which would enable us to simultaneously identify and quantify H. filipjevi from infested soils.

**Effect of UV-A and UV-B on airborne conidia concentrations of Erysiphe necator in Eastern Washington**

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Phytopathology 101:S196

To characterize the relationship between UV radiation and populations of airborne conidia of Erysiphe necator in grape vineyards in Eastern Washington, conidia were trapped under natural conditions using a Burkard volumetric air sampler positioned in a vineyard near Prosser, WA during the...
2008 and 2009 growing seasons. Time series cross correlation analysis was selected as an appropriate technique to measure the lagged relationship between UV radiation series and conidia series. The results of this study described the negative relationship of UV radiation series data with conidia population series in current day (day0) and the previous two days (day–2). The cross correlations of UVA with conidia concentrations were –0.306 in lag 0 and –0.182 in lag 2, and that of UBV with conidia were -0.311 in lag 0 and –0.235 in lag2 based on prewhitening series. Under natural conditions, low levels of UV radiation resulted in higher population of E. necator conidia, while high UV had the opposite effect. The thresholds of positive and negative effects of UBV and UVA were 0.4-0.6 W/m² and 11-15 W/m², respectively. However, effects of UV radiation on conidia can be influenced by temperature and relative humidity. Temperatures of 21–27°C can lowered the impact of UV radiation on conidia concentrations while high levels of relative humidity intensified the effect of UV radiation.

The time lagged effects on the relationship between weather variables and airborne spore concentration of Erysiphe necator

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Phytopathology 101:S197

Conidia of Erysiphe necator were collected by Burkard volumetric sampler for developing spore concentration prediction models in Prosser, WA during 2008 and 2009. The model used spore population and meteorological parameters as independent predictors. Two time series analysis approaches (ARIMA and PDLREG) were applied to quantify that spore concentration was explained by previous values and weather variables. The current value of concentration was related to previous values, autocorrelations in this series were 0.918-0.870. ARIMA (4.0.0) model were appropriate to characterize the association between the current value and lag 1 to lag 4 values in concentration series. Polynomial distribution lag regression model described the delayed effect of weather variables on spore concentration during 7 days at polynomial degree of 2 (l = 2). Temperature related weather variables and average dew point were better predictors than average relative humidity, average leaf wetness and daily rainfall. Temperature duration variables were superior to average air temperature, maximum air temperature and minimum air temperature. This study suggests that the delayed effect of weather variables and previous values of conidia concentration can improve the forecasting of conidia concentration.

Identification of type III secretion inhibitors in Erwinia amylovora, the causal agent of fire blight of apple and pear

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Phytopathology 101:S197

The type III secretion system (T3SS) is a potent virulence mechanism shared by a broad spectrum of gram-negative bacteria that infect both plant and mammalian hosts. T3SS encoded proteins are injected into plant host cells, thus manipulating the host immune response. It is reasonable to believe that disabling the T3SS function may provide another way of controlling bacterial diseases. High-throughput screening of chemical libraries have identified small molecule inhibitors that attenuate T3SS of mammalian pathogens, but no report so far for plant pathogenic bacteria. In this study, using an in vitro reporter, we screened and identified five chemicals that suppressed T3SS gene expression of Erwinia amylovora and three chemicals that delayed hypersensitive response (HR) in tobacco. One chemical was further characterized by conducting global gene expression assay with E. amylovora grown in hrp-inducing minimal medium treated with chemicals. Our results showed that majority of genes in E. amylovora T3SS pathogenicity islands including hrp, as well as several effectors including avrRpm2, hrcC were down-regulated more than two fold. Surprisingly, expression of amylovoran biosynthesis genes was also suppressed, whereas siderophore biosynthesis genes were strongly induced by the chemical. These findings indicate that inhibitors against T3SS without interfering bacterial growth could be explored for controlling bacterial diseases.

Identification and pathogenic analysis of Colletotrichum species causing soybean anthracnose

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Anthraxcon of soybean [Glycine max (L.) Merr.] is known to occur wherever soybeans are grown. The disease seriously affects agricultural economies especially in wet, warm and humid areas. The most common pathogen that causes soybean anthracnose is the fungus Colletotrichum truncatum. Several other Colletotrichum species have also been reported, including C. coccodes, C. destructivum (teleomorph, Glomerella glycinonis), C. gloeosporioides (teleomorph, G. cingulata), and C. graminicola (teleomorph, G. graminicola). It is important to screen, isolate and identify the Colletotrichum species that cause soybean anthracnose to study the disease epidemiology and to develop resistant soybean genotypes. In this study, we collected more than 80 Colletotrichum isolates from infected soybean petioles from different states and initially identified them by size and shape of conidia and appressoria and the teleomorphic state. Among them, 57 were curved-spored types and the rest were straight-spored types. In addition, molecular methods such as PCR combined with multi-gene phylogenetic analysis will be applied and compare to the results of morphological observations. Furthermore, isolates were tested for their pathogenicity on soybean cultivars. There were differences in isolates in their capacity to produce symptoms on soybean.

Fungi and oomycetes associated with a peach replant problem

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Phytopathology 101:S197

The objective of this study was to identify fungi and oomycetes associated with a peach replant problem at a field location at the UC Kearney Agricultural Center in California. Soil samples were exposed to a constant water bath temperature between 40 to 70°C and held for 30 minutes once the center of the sample reached the target temperature. Peach seedling were transplanted into the treated soils and grown for 6 weeks in a greenhouse. Plant growth parameters were measured while fungal and oomycete composition was analyzed by culture and culture-independent methods. An increase in seedling growth correlated with the soil treatment temperature. Trichoderma asperellum, Trichoderma virens and Fusarium oxysporum were the most abundant fungal strains isolated. Sequence-selective quantitative PCR analyses of these fungi showed that there were no significant associations between Fusarium oxysporum and plant growth parameters, yet Trichoderma species were associated with increased peach seedling growth. Culture-independent analyses identified a significant negative association between Pythium vexans and plant growth, indicating that it may be contributing to the replant problem.

Early warning method and system for cucumber diseases in solar greenhouses

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Phytopathology 101:S197

The disease early warning is an essential way for protecting eco-environment and improving the level of vegetable quality safety in solar greenhouses. We studied the early warning method and system in solar greenhouses with four parts as follows. Firstly, we combined the leaf wetness sensors and estimation method of leaf wetness duration (LWD) with the T3SS gene expression and the leaf wetness duration (LWD) monitoring method. The errors were around 1–2 h; compared with the LWD that was over 3 h, the monitoring effects of the method were acceptable. Secondly, we developed the primary infection situation early warning models of important diseases, such as cucumber downy mildew (Pseudoperonospora cubensis), in solar greenhouses. The years were evaluated by over a 4-year (2005–2008) over the whole field. The results showed that it could warn the primary infection and disease occurrence date with a probability of 95% and more than 2 days before disease appearance. Thirdly, considering the characteristics of multi-warning sources of cucumber downy mildew in solar greenhouses, the warning source traceability model for cucumber downy mildew in solar greenhouses was constructed for system ease-realization using chain-style theory of disaster. At last, the cucumber disease early warning system in solar greenhouses was developed by Visual Studio 2005 and SQL Server 2000, which added more functions of ongoing decision making for revising cultural practices than traditional record-keeping systems. The method and system shows promise for increasing adoption for IPM in China, and can provide decision support for early warning of cucumber diseases in solar greenhouses.

Forest products protection: From chemical to biological roadways

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Phytopathology 101:S197

Wood is a renewable resource and plays an important role in the world economy; however, it is subject to attack from wood-degrading fungi and insects. Developing effective and low environmental impact technologies for wood pest control is a big issue in forest products industry. During the past years, various chemical products have been used as wood preservatives such as cresole, pentachlorophenol (PCP) and chromated copper arsenate (CCA).
In the current years, less toxic copper, boron-based biocides are mostly used for protection of wood products. At the same time, organic biocides have been developed and put in the market, and more and more chemical- or thermal-modified wood products have been available for certain use in building construction. For the future development, more studies are focused on biological ways in wood protection such as utilization of extractives from natural durable plants as biocides, biological protection of wood against insect, stain and decay damage, and genetic engineering trees for wood pest resistance. This presentation will review the history of using chemical biocides in wood protection and will discuss the challenges and future directions in the development and the use of biological products or process in forest products industry.

PCGI encodes a novel splicing factor that is essential for pathogenesis in Magnaporthe oryzae

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Phytopathology 101:S198

PpPr19 and its associated proteins play vital roles in assembly and disassembly processes of spliceosomes. In this study, PCG1, a novel gene essential to pathogenicity of Magnaporthe oryzae was identified by insertion mutagenesis. PCG1 encodes a protein with similarity to HCCDC12, a component associated with hPpPr19 in Homo sapiens. Deletion of PCG1 resulted in severe defects in asexual development and loss of pathogenicity. Pcg1 protein was expressed throughout the infection processes and localized to the nucleus. Forty-six nuclear proteins were identified to be communoprecipitated with Pcg1, more than 20 of which are similar to well-known components of spliceosomes. RNA-seq analyses revealed that intron retention is the major defect in the Δpcg1 null mutant. MoPFF3, a novel pathogenicity gene was identified among 421 genes, whose transcripts were not fully spliced in the Δpcg1 mutant. Interestingly, both Pcg1 and hCcdc12 could physically interact with MoCwc4 and its counterpart HcRN1 from H. sapiens. Constitutive expression of hCcdC12 could partially complement defects of the Δpcg1 mutant. In addition, FgPeg1, Peg1 ortholog in Fusarium graminearum, was also required for asexual development and pathogenicity, and these two genes could be functional interchangeable between the two fungi. Our data indicated that Pcg1 is a novel splicing factor controlling intron splicing efficiency of premRNAs and plays vital roles in infection-related morphogenesis in pathogenic ascomycetes.

A critical amino acid of 6K2 protein of Papaya ringspot virus for inducing wilting symptom on papaya plants

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Phytopathology 101:S198

The isolates of Papaya ringspot virus (PRSV), the major limiting factor for papaya production in tropical and subtropical areas, are classified as P-type (papaya-infecting) or W-type (non-papaya-infecting), according to their host papaya production in tropical and subtropical areas, are classified as P-type virus. Most strains of P-type virus induce mosaic symptoms on papaya, (papaya-infecting) or W-type (non-papa ya-infecting), according to their host plant. The isolates of Papaya ringspot virus (PRSV) W-CI6K caused wilting symptom similar to the naturally occurring P-type PRSV isolate, W-CI 619. To investigate the determinant for wilting, further research was conducted from a 619bp fragment of the internal transcribed spacer region (ITS) of the 16S-23S rDNA of A. avenae. A. avenae subsp.avenae has been described from oat, barley, and rye, but there was no significant difference in the pathogenicity of the two strains. A. avenae subsp.avenae has been described from oat, barley, and rye, but there was no significant difference in the pathogenicity of the two strains. A. avenae subsp.avenae is the preferred method to control the disease, but the effectiveness of race specific resistance is typically not durable due to the genetic plasticity of rust populations. In this study, we identified a Pst effector (PSTeTr) that was highly expressed in haustoria. The PSTeTr gene delivered by modified Pseudomonas fluorescens strain E7rN into leaves of wheat cultivar Tres, which has resistance genes YrTr1 and YrTr2, induced noticeably more callose depositions than the empty pEDV6 vector and the vector inserted with other two Pst genes (PuthA25 and PsthA5A23). Sensing PSTeTr in leaves of Tres through BSMV-VIGS produced abundant uredinia in most leaves inoculated with a Tres-avirulent race, while the leaves infected by BSMV::MCS with the control constructs with no Pst gene and with PsthlA203 sporulated sparsely. In contrast, silencing PSTeTr in other resistant wheat cultivars with Yr15 or Yr10 did not change the resistance reactions. The results indicate that PSTeTr is required for avirulence on wheat cultivars.

Detection and damage analysis of Acidovorax avenae subsp.avenae in proso millet

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The bacterium, Acidovorax avenae subsp.avenae causes several important plant diseases including bacterial stripe of rice, bacterial stalk rot of corn, bacterial leaf blight of oats, and red stripe of sugarcane and millet. Three sets of qRT-PCR primers for detection of quorum-sensing and virulence genes in proso millet were designed from a 619bp fragment of the internal transcribed spacer region (ITS) of the 16S-23S rDNA of A. avenae subsp.avenae strain MY1. Investigated a growth difference for the 7-days, 14-days, and 21-days seedling inoculated the A. avenae subsp.avenae, but there was no significant differences in inoculation time. However in proso millet variety test, they show difference infected kernel rate, and seed weight.

Roles of NtERF5 in N-gene mediated TMV resistance

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Phytopathology 101:S198

Resistance in tobacco to Tobacco mosaic virus (TMV) mediated by the N gene requires a hypersensitive response (HR) which prevents further spread of TMV from infected cells. Over-expression of the la protein of Cucumber mosaic virus in N-gene tobacco neutralized a novel resistance specific to TMV-GFP and ethylene-response factor 5 (NtERF5), which interacted with the la protein in the yeast two-hybrid system, played a crucial role in the defense mechanism against TMV spread. NtERF5 expression was controlled by a salicylic acid (SA)-independent pathway of the N gene resistance. We also found that NtERF5 interacted in the yeast two-hybrid system with the tobacco Myb1 gene, which also was induced by TMV during the HR. NtERF5 and...
Myb1 co-localized in the nucleus and on tonoplast membranes in Nicotiana benthamiana cells using confocal microscopy. We generated NiER5S and/or Myb1- singly or doubly silenced transgenic tobacco. Interestingly, TMV could move systemically to upper leaves in NiER5S-silenced tobacco plants. TMV moved much faster to upper leaves of NiER5S/Myb1-doubly silenced tobacco plants at 25°C while TMV was restricted to the inoculated leaf tissues of Myb1-silenced tobacco plants. Taken together, these results from transgenic studies supported strongly the conclusion that NiER5S is a novel protein in the SA-independent pathway of the N gene resistance mechanism and associates with the tobacco Myb1 gene in TMV resistance.

**BBWV2-resistant transgenic Nicotiana benthamiana expressing a virus-derived hairpin RNA**

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Phytopathology 101:S199

Broad bean wilt virus 2 (BBWV2), one of the important pepper-infecting viruses, causes economically severe loss of pepper production in Korea. BBWV2 consists of a single-stranded, positive sense RNA, bipartite genome and is taxonomically classified in the genus Favivirus (the family Comoviridae). Sequence alignments showed highly conserved sequences in 5′ non-coding regions (NCRs) of RNA1 and RNA2 in all BBWV2 strains characterized so far. Based on this observation, we constructed transgenic Nicotiana benthamiana plants expressing an inverted repeat harboring a 210 bp cDNA fragment of the conserved 5′ NCR of BBWV2. The transgenic N. benthamiana plants inoculated with BBWV2 did not produce any symptoms in non-silenced plants, whereas BBWV2 RNA1 and RNA2 were detected in transgenic N. benthamiana plants. Accumulation of virus-derived small interfering RNAs was detected in the inoculated leaf tissues of the transgenic N. benthamiana plants, indicating RNA silencing is responsible for the resistance to BBWV2.

**Identification of a candidate resistance gene to Phakopsora pachyrhizi, the causal agent of soybean rust, in the alternative host kudzu, Pueraria spp.**

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Phytopathology 101:S199

Soybean rust (SBR), caused by the biotrophic fungus Phakopsora pachyrhizi, is a potentially destructive disease to U.S. soybean (Glycine max) production. The fungus over-winters in the south eastern U.S. on exotic, invasive kudzu species (Pueraria spp.), providing initial inoculum for each season that has the potential to disperse to northern soybean producing areas. A more precise understanding of resistance in kudzu can improve estimates of initial inoculum for forecast models. Five major sources of SBR resistance have been identified in soybean, Rpp1 to Rpp5. A resistance candidate gene, Rpp4C, belonging to the CC-NBS-LRR gene family has been associated with Rpp4 resistance. We predicted SBR resistance in kudzu also might be associated with Rpp4C orthologues, in particular for a kudzu accession (FLAL15), which shows a SBR resistance reaction similar to Rpp4. Primers targeting the conserved NBS and LRR domains of the soybean Rpp4C resistance gene were used to amplify Rpp4 candidate gene orthologues from our S. chinensis and S. austus accessions. Amplicon analysis revealed multiple sequence fragments for at least 5 Rpp4-orthologue candidate genes, named Rpp4K1 to Rpp4K5, sharing from 85% to 91% nucleotide identity to the Rpp4C genes. Efforts to demonstrate association between the genotypic profile of each kudzu accession with the SBR susceptibility phenotype are being pursued.

**Engineering an infectious cDNA clone of an Arizona Pepino mosaic virus isolate**

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Phytopathology 101:S199

Citrus huanglongbing (HLB), associated with ‘Ca. Liberibacter asiaticus’ (CaLas) is the most serious disease of citrus in Florida and Brazil. Antibodies are the most widely used tool to detect pathogens, and they are also uniquely useful as experimental reagents. Psyllids that contained more than 10³ CaLas were used to immunize BALB/C mice. mRNA from the spleens of the immunized mice was purified and converted into cDNA library. Antibody gene repertoire were PCR-amplified using 23 primers for the heavy chain variable region (VH) and 21 primers for the light chain variable region (VL). The VH and VL were joined by overlap extension PCR, and the completed scFv inserts were ligated into the phage vector pKM19. Forty-five clones were picked at random from the library and tested by PCR. All tested clones contained scFv inserts of about 750 bp. The BstN1 fingerprints of 23 scFvs randomly selected inserts were each unique and we estimate that the library contains 1.3 × 108 independent clones with full-length scFv inserts. Several positive clones were expected to be exposed on the bacterium’s surface were expressed, purified, and used as capture antigens. ELISA data show that several different scFvs with specificity for different CaLas proteins were obtained. These include the enzyme producing polysaccharide capsule polysaccharidic acid (PSA), a component of a type IV pilus (TFP), and the major outer membrane protein (OMP66). Screening is in progress with other antigens. The utility of these selected antibodies will be discussed. Reduced infection of wheat spikelets inoculated with ascosporae of Gibberella zeae in the presence of fungal mating pheromone peptides G. Y. Yuen (1), C. C. Jochum (1), N. W. Gross (2), T. J. English (2), J. F. Leslie (3)
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Phytopathology 101:S199

Anti-fungal peptides are an emerging area of antibiotic therapy with application for control of wheat head blight, caused by G. zeae. In past
The inhibition of the seed borne pathogenic fungus *Aspergillus niger* that causes root rot diseases in peanut was investigated using soil-isolated Plant Growth Promoting Rhizobacteria (PGPR) as biological controllers. The best 4 PGPR isolates that were selected were A20, A45, A62, and A106. The sequence of 16S rDNA genes of these selected strains indicated that A20, A45, A62, and A106 were highly homology to *Bacillus megaterium* strain AM1C7 (99%), B. subtilis strain Setapak 8 (99%), B. subtilis subsp. subtilis strain SB 3130 (99%), and *Pseudomonas* sp. NJ-61 (95%), respectively. The strains A20, A45, A62, and A106 were able to inhibit *A. niger* growth at 42.5%, 51.42%, 67.81%, and 44.53%, respectively. Antifungal activities were found clearly in cell-free supernatants of A20 and A62. Interestingly, the antifungal activity of isolates A45 and A62 was proteinase k resistant. This implied that the mode of action against the fungus from these isolates was not from protease enzyme. All of the PGPR isolates could produce indole-3-acetic acid (IAA), an auxin hormone. IAA hormone produced from PGPR isolates could promote peanut root growth. When either isolate A20 or A45 (10^6 cells per ml) was co-inoculated with *Bradyrhizobium* sp. TAL 173 (10^6 cells per ml), the peanut root disease caused by *A. niger* (10^3 and 10^6 spores per root) could be inhibited. Therefore, application of co-inoculum of rhizobia-PGPR is likely a viable agricultural technology to increase nitrogen fixation and reduce fungicide usage.

**First report of a bacterial disease in Australian cedar (Toona ciliata)**


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Phytopathology 101:S200

The aim of this study was to isolate and identify the causal agent of a foliar disease of Australian cedar (*Toona ciliata*), which is important due to the physical characteristics of the wood and is found in nurseries in Brazil. Exudation test, isolation, pathogenicity test and resolation were performed, fulfilling Koch’s postulates. The isolates were subjected to biochemical tests to determine the genus of the pathogen. Molecular identification of the genus of bacteria consisted of the amplification of genomic fragments of approximately 1400 bp, using primer ERIIC by polymerase chain reaction (PCR), sequencing and phylogenetic analysis. The isolated colonies were whitish slimy-looking. The bacteria were Gram negative, strictly aerobic. The utilization of carbohydrates and amino acids was determined. They produced fluorescent pigments on King B medium. They were positive for glucose as carbon source, gas production, and starch hydrolysis. The bacteria induced HR on tomato and pepper. The comparison of the DNA sequence of the isolates with those available in GenBank indicated that the bacterium in question in the genus *Xanthomonas*, but is not identical to any known species.

**The effect of phase variation on the interaction of Salmonella enterica sv. Typhimurium with tomatoes**

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Phytopathology 101:S200

Several recent outbreaks of gastroenteritis have been linked to non-typhoidal Salmonella within fresh fruits and vegetables. For interactions with plants, Salmonella relies on an array of surface structures. Phase variation is thought to regulate the production of these surface structures and has been associated with increased virulence in Salmonella. We hypothesize phase variation may affect the ability of the bacterium to persist in plants. We assayed the attachment of wild type Salmonella enterica sv. Typhimurium and its phase variants to tomato surfaces. A cellulose-deficient mutant and high frequency phase variant attached to tomato surfaces at lower rates than the wild type that consistently produces extracellular polymers. Using RIVET, the roles of two surface structures, the production and assembly of fimbriae, were studied. Attachment fimbriae structures were tested inside tomatoes. yih operons are involved in capsule assembly and translocation. agB is a curlin nucelator protein involved in fimbriae assembly. RIVET reporter constructs in yihT and agB in both wild type and phase variant backgrounds were infected into tomatoes and recovered after a week. The expression of yihT was increased in high-frequency phase variants compared to wild type. In the low-frequency phase variants, reduced level of expression of both yihT and agB genes compared to wild type was observed. These results will be investigated to determine if this difference in expression confers an advantage to survival within tomatoes.

**Incorporation of peanut rhizobia with plant growth promoting rhizobacteria as biocontroller effectively against the seed borne fungi, *Aspergillus niger***

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Phytopathology 101:S200

Research we demonstrated that mating pheromone peptides derived from *G. zeae* and other ascomycete fungi, and combinatorially selected peptides can inhibit or disrupt germination of pathogen ascospores. We are now assessing the potential for mating pheromone and combinatorial peptides to protect wheat spikelets from infection. For assessment, peptides were synthesized and applied to individual spikelets in combination with a water droplet containing infectious spores. In initial experiments, only 1% of spikelets became infected in the presence of 20 µM Pgz, the mating pheromone derived from *F. graminearum*. Pathogen mycelial growth on spikelets was also severely reduced at this peptide concentration. A representative combinatorial peptide also significantly reduced spikelet infection. Protective effects of all peptides declined with decreasing concentration. We are currently evaluating the protective efficacy of additional mating pheromone peptides and their derivatives when compared to Pgz as a standard treatment. Assessments are being made over a range of concentrations to identify the best peptides for larger-scale greenhouse trials. If effective, inhibitory peptides could be applied as a protective spray to wheat during flowering or alternatively, deployed in transgenic wheat.

**Biological control of Fusarium head blight in wheat caused by Gibberella zeae - a two-year, multi-location study**


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Phytopathology 101:S200

Biological agents for controlling Fusarium head blight (scab) and deoxynivalenol (DON) accumulation in grain are needed to augment host resistance and chemical fungicides or to serve as the primary management tool where the other strategies are not available. Field experiments were conducted in 2009 and 2010 at six sites to evaluate two biocontrol materials, one being a mixture of two yeast strains, *Cryptococcus flavescens* OH 182.9 (NRRL Y-30216) and *C. aureus* OH 71.4 (NRRL Y-30213), that were co-cultured in the same fermentor. The other was Taegro, a commercial product containing *Bacillus anomyloficiens* FZB24. The biocontrol materials were tested as stand-alone treatments or applied in combination with a commercial fungicide Prosaro 421 SC (prothioconazole plus tebuconazole). When applied alone, the biocontrol reduced scab levels relative to the control in some individual experiments and averaged across experiments. The biological treatments, however, were not as efficacious as the fungicide alone. Although the various biocontrol-fungicide combinations did not confer any significant advantage over a single fungicide application in regard to controlling scab, applying either biocontrol material as a sequential treatment several days after the fungicide was more effective than the fungicide alone in reducing DON content in the harvested grain. Presumably, this advantage was due to the biological agents inhibiting late infections.
Entomopathogenic nematodes (EPNs) are parasitic natural enemies of many agricultural pests which are widely used as biological control agents. They can suppress or evade the host immune defense upon entry into insects. The surface coat of Steinernema glaseri was proved to play important roles in defeating the host immune system. In this work, a protein fraction with immunosuppressive activity was separated by electro-elution and further analyzed with 2D-electrophoresis. LC-MS analysis of protein spot from 2D-electrophoresis gave five peptides. Based on the sequences, specific primers were designed and the full-length cDNA sequence of the encoding gene was cloned. The deduced protein, Sg-E1, was then expressed in E. coli. Using immuno-gold transmission electron microscopy, native Sg-E1 was confirmed to be located on both nematode cuticle and surface coat. Furthermore, Sg-E1 was detected in host hemolymph after infection of Galleria mellonella with S. glaseri, indicating that Sg-E1 was secreted into insect hemocoel and involved in infection. This is the first report of the cloning and characterization of a surface coat protein in EPNs. Our findings help to illuminate the mechanism of EPNs defeating the host immune system.

**Genome and transcriptome analysis of Geosmithia morbida**


Thousand canker disease of black walnut is the result of aggressive feeding by the walnut twig beetle (Pityophthorus juglandis) and canker formation around galleries caused by Geosmithia morbida. The disease is widespread in the western United States and was detected in the native range of black walnut in Tennessee in 2010. G. morbida is the first phytopathogenic species reported in this genus and in the Bionectriaceae. We started a genome and transcriptomic analysis of G. morbida to better understand its genome complement and for comparison of its genome to other fungal pathogens in the Hypocreales. Total DNA of isolate CBS124663 was sequenced in the yeast phase. Genome sequencing resulted in 779,553 index Reads that assembled into 27,933 contigs. This represented 16 million of non-redundant base pairs, or approximately 1/3 of the predicted genome size. A total of 15,254 sequences (7,697 contigs and 7,557 singletons) were automatically annotated using the Blast2GO similarity tool (http://www.blast2go.org). Another 3.4 Mbp of non-redundant sequences of the fungus transcriptome were assembled into 5,773 contigs and that were subsequently mapped into 6,867 contigs of the G. morbida genome.

**Population structure of Geosmithia morbida in the United States is complex**

M. M. ZERILLO (1), K. Woeste (2), E. Freeland (1), S. Seybold (3), W. Cranshaw (1), N. Tisserat (1)


Thousand Canker Disease of black walnut, the causal agent of the disease, is confirmed to be located on both nematode cuticle and surface coat. Canker formation around galleries caused by Geosmithia morbida. The disease is widespread in the western United States and was detected in the native range of black walnut in Tennessee in 2010. G. morbida is the first phytopathogenic species reported in this genus and in the Bionectriaceae. We started a genome and transcriptomic analysis of G. morbida to better understand its genome complement and for comparison of its genome to other fungal pathogens in the Hypocreales. Total DNA of isolate CBS124663 was sequenced in the yeast phase. Genome sequencing resulted in 779,553 index Reads that assembled into 27,933 contigs. This represented 16 million of non-redundant base pairs, or approximately 1/3 of the predicted genome size. A total of 15,254 sequences (7,697 contigs and 7,557 singletons) were automatically annotated using the Blast2GO similarity tool (http://www.blast2go.org/). Another 3.4 Mbp of non-redundant sequences of the fungus transcriptome were assembled into 5,773 contigs and that were subsequently mapped into 6,867 contigs of the G. morbida genome.

**virulence** and **molecular comparison of Puccinia striiformis f. sp. tritici populations in China and the United States**


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Phytopathology 101:S201

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), is one of the most important diseases of wheat in both China and the U.S. The *Pst* populations of these countries were compared for virulence patterns on wheat genotypes used to differentiate races of the pathogen, and genotypes using simple sequence repeat (SSR) markers. From 86 Chinese isolates, 58 races were identified based on reactions on the 17 Chinese differentials and 52 races were identified based on the 20 U.S. differentials. The selected 51 U.S. isolates, representing 50 races based on the U.S. differentials, were identified as 42 races using the Chinese differentials. A total of 132 virulence patterns were identified from the 137 isolates based on their reactions on both Chinese and U.S. differentials. From the 137 isolates, SSR markers identified 102 genotypes, of which 71 from the Chinese isolates and 31 from the U.S. isolates. Virulence and SSR data had a low (r = 0.38), but significant (P = 0.01) correlation. The Chinese and U.S. populations had similar levels of diversity based on Kosman indices. Principal analysis using the SSR data separated the two populations more clearly than using the virulence data. A non-rooted tree generated using the molecular data indicated that the Chinese and U.S. populations have diverged independently, but may share the same origin, which was also supported by the low value (0.13) of differentiation and high value (1.74) of gene flow.

**Construction of recombinant fluorescent Pseudomonas spp. for suppression of soilborne pathogens**


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Phytopathology 101:S201

Take-all, caused by Gaumannomyces graminis var. tritici, and Rhizoctonia root rot, caused by *R. solani* and *R. oryzae*, are among the most important soilborne diseases of wheat in the Pacific Northwest. Because of the lack of resistance to these and many other soilborne diseases, wheat roots rely on antagonistic rhizosphere microorganisms as a first line of defense against these diseases. Many of these antagonists lack activity against a wide range of pathogens. The purpose of this study was to construct recombinant fluorescent *Pseudomonas* spp. that produce multiple antibiotics and to determine their activity against soilborne pathogens. We stably inserted the biosynthesis loci for different antibiotics into various biocontrol strains of *P. fluorescens*. All recombinant strains produced both their indigenous antibiotic and that encoded by the introduced genes, but the level of antibiotic production varied significantly depending on the transgenes introduced and the recipient *P. fluorescens* strain. In general, recombinant strains inhibited target pathogens better than the respective wild-type strain, but inhibition varied by the strain. Our results indicate the need to carefully screen strains and antibiotic combinations to obtain recombinants that retain the traits of the parental strain and exhibit broader activity due to the transgenes.

**Further spread of and domination by Bemisia tabaci biotype Q on field crops in China**

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Phytopathology 101:S201

The sweetpotato whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), causes severe crop losses to many crops. The worst of these losses are often associated with the invasion and establishment of biotypes B and Q of this pest. The previous research in 2007 showed that biotype Q was less virulent than other biotypes in field populations in China. To know about the current status of the biotypes composition in the field, an extensive survey covering mainly eastern parts of China was conducted in 2009 to determine the current distribution of B. tabaci biotypes. Using PCR primers specific for the mtCOI (mitochondrial cytochrome oxidase I) of biotypes B and Q and gene sequencing, we determined the biotypes composition in 61 whitefly populations and their distribution across 19 provinces in China. Our research revealed that only biotypes B and Q have been found in the field in 2009 in China. Among them, biotype Q was dominant in 44 locations.
(100.0%) and biotype B was dominant in 17 locations (100.0%). The current survey indicates that biotype Q has rapidly displaced biotype B in most locations in China.

What we can learn from high similarities of molecular mechanisms between barley host and nonhost resistances to Blumeria graminis T. Zhang (1), J. Huan (1), J. Huang (1), J. Shi (1), M. Cheng (1), W. Kuang (1), W. DONG (1)

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PEOPLES REP OF CHINA

Phytopathology 101:S202

Nonhost resistance is strong, effective, and durable. Powdery mildew pathogens of Blumeria graminis f. sp hordei (Bgh, adapted to barley) and B. graminis f. sp. triticici (Bgt, nonadapted to barley) are ideal systems to explore the mechanisms of nonhost resistance. We inoculated barley seedlings with Bgt and Bgh, separately. Leaves were collected at 0, 6, 12, 24, 36 hours after inoculation. We compared expression profiles of barley host and nonhost resistances based on 849 regulated genes. Expression patterns between host and nonhost resistances are similar, especially at time points of 24 and 36 hours, which the correlation coefficient indexes are 0.9322 and 0.9474, respectively. Based on our microarray data, we selected 389 up-regulated genes and tested their resistances to Bgh and Bgt by transient induced gene silencing. We found that 16 genes are resistant significantly to both Bgh and Bgt, but no gene is specifically resistant to Bgt only. This is not surprising because most reported mechanisms of nonhost resistance are overlapped with that of host resistance. Question is that the overlapped mechanisms cannot explain the distinct differences between host and nonhost resistances. So, we infer that the components of nonhost resistance are possibly constitutively expressed or slightly induced but non-detectable in our array experiments. Currently, we are systematically screening the major components of nonhost resistance in barley and wheat by high-throughput yeast hybrid protein-protein interactions.

Evaluation of chemicals for control of citrus canker, Xanthomonas citri subsp. citri

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Phytopathology 101:S202

Citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), is one of the important diseases of citrus worldwide. Selected chemicals were evaluated for control of Xcc on citrus in greenhouse with three replicates using a randomized complete block design. Two antibiotics (oxytetracycline and streptomycin), one Bacillus subtilis (BS) extract, one copper-based bactericide and salicylic acid were sprayed three times at one-week intervals before or after inoculation of Xcc on grapefruit leaves. Each chemical/extract was tested twice with 10 to 15 leaves on one branch per treatment. Xcc colony-forming units (CFU) and bacterial titer were quantified from a single lesion of each treatment. Oxytetracycline, the copper-based bactericide and the BS extract consistently inhibited the bacterial growth, decreased CFU by 88.3%, 1.81% and 74.2%, respectively, when compared to the water-treated control. The effectiveness against Xcc bacterium was 4-fold increase with the spray applications of these chemicals before versus after Xcc-inoculation. Oxytetracycline was better than streptomycin or copper-based bactericide for the control of citrus canker. The Xcc bacterium did not multiply in citrus leaves pre-treated with oxytetracycline before Xcc-inoculation. Adequate application of effective chemicals, such as oxytetracycline may successfully control citrus canker.

Assessment of copper resistance in populations of Pseudomonas syringae pv. phaseolicola, the causal agent of halo blight on snap bean

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Phytopathology 101:S202

Halo blight infects foliage and pods of beans, and is a major bacterial disease on beans worldwide caused by Pseudomonas syringae pv. phaseolicola (Psp). Psp is most destructive in areas such as Florida where temperatures are moderate to high. A novel DNA virus was discovered in gromvive upon deep sequencing of cDNA libraries that were constructed from small RNAs of grapevines. Results from polymerase chain reaction (PCR) assays indicated that the DNA virus was closely associated with the vein clearing and vein decline syndrome that affects vineyards in the Midwest U.S.A., and thus is given a provisional name Grapevine vein clearing virus (GVCV). Three overlapping DNA fragments were amplified from the symptomatic vine and subject to sequencing from both directions by primer walking. The whole genome of GVCV was assembled and represents a circular DNA of 7,752

Analysis on population sources of the first generation Loxostegis sticticalis L. (Lepidoptera: Pyralidae) moth in China

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PEOPLES REP OF CHINA

Phytopathology 101:S202

The beet webworm moth, Loxostegis sticticalis L. (Lepidoptera: Pyralidae), is a worldwide pest mainly distributing on the Eurasia Continent and North America. Its first generation outbreak in 2008 and caused massive losses to the farming and animal husbandry industries in China. The migration behavior of the insect pest were observed with a vertical-looking radar, a simultaneously-operated searchlight trap and surface light trap at the radar station in Xilinhot, Inner Mongolia Autonomous Region. The population sources were studied by the means of wind field analysis and trajectory calculation. The results showed that the first generation moths in North and Northeast China at the end of July mainly came from the central and east regions of the Republic of Mongolia, the China-Mongolia and China-Russia border. The peaks of the moths occurred in most regions of North China at the early August were mainly caused by the moths dispersed by the north–easterly and south-eastern airflows. The moths occurred on 6 August at Heilongjiang Province mainly originated from Russia and the China-Russia border. Therefore, it is an effective way to control the international pest by strengthening the cooperative with Mongolia and Russia and to study on the migration behavior and population sources of the L. sticticalis moth in large-scale.

Evolution of mode of infection in the rice blast fungus and allied species

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Phytopathology 101:S202

The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as Magnaporthe oryzae (rice blast fungus, formerly known as M. grisea), M. poae (summer pathogen pathogen of turf grasses), and Gaumannomyces graminis (take-all fungus of various cereals and grasses), which are popular model organisms in fungal biology and host–pathogen interaction studies. Despite their ecological and economic importance, the phylogenetic relationships among the constituent species remain ambiguous due to the lack of convincing morphological characters and paucity of molecular data for the majority of the non-model species in the family. In this study, our multilocus phylogeny suggests that both Magnaporthe and Gaumannomyces are polyphyletic genera. The phylogeny also provides insights into fungal biology and pathogenesis. Magnaporthe oryzae forms a basal clade, while M. poae and M. rhizophila formed another well-supported clade with G. incrustans, G. graminis and M. salvinii. The basal species infects both root and aerial parts of plant host, while the aerial infection capacity seems to be lost in the taxa of the latter clade. The study indicates that anamorphic and ecological features are more informative than the teleomorphic characters in defining monophyletic groups among these taxa.

A DNA virus in grapevine and its association with vein-clearing and vine decline syndrome

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Phytopathology 101:S202

More than sixty viruses that are known to infect grapevine all contain RNA as their genetic material. A novel DNA virus was discovered in gromvive upon deep sequencing of cDNA libraries that were constructed from small RNAs of grapevines. Results from polymerase chain reaction (PCR) assays indicated that the DNA virus was closely associated with the vein clearing and vine decline syndrome that affects vineyards in the Midwest U.S.A., and thus is given a provisional name Grapevine vein clearing virus (GVCV). Three overlapping DNA fragments were amplified from the symptomatic vine and subject to sequencing from both directions by primer walking. The whole genome of GVCV was assembled and represents a circular DNA of 7,752
base pairs. Open four reading frames (ORFs) are predicted on the sense genomic strand. The sequence of GVCV is most closely related to the genomes of badnaviruses in the family Caulimoviridae. GVCV has been detected in six grape varieties that show similar syndrome in Missouri, Illinois and Indiana. Restriction Fragment Length Polymorphism (RFLP) and sequencing of DNA fragments revealed a great genetic diversity of GVCV populations in commercial vineyards. Interestingly, GVCV was also found in wild grapevine species in their native habitats in Missouri. Discovery of this novel DNA virus allows us to investigate its epidemics and damages to the grape production, and its causal relationship with the syndrome.

Interactions of post emergence herbicides, strobilurin fungicides, and Rhizoctonia root rot of soybean

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Phytopathology 101:S203

Rhizoctonia root rot, caused by Rhizoctonia solani is a major disease of soybeans in the north-central United States. The emergence of glyphosate resistant weeds and the payment premium for growing non-GMO soybeans has increased the use of non-glyphosate herbicides on soybeans. However, some of these herbicides may increase the severity of root rot caused by Rhizoctonia solani. Field and greenhouse studies were conducted to evaluate the potential interaction among glyphosate-tolerant soybeans, post emergence herbicides (glyphosate, acifluorfen, lactofen, imazethapyr), and fungicide seed treatments (azoxystrobin, pyraclostrobin, trifloxystrobin). Inoculum of R. solani (AG 2-2) was planted along with the treated seed. Herbicides were applied to field plots at the V4 growth stage. Plant stand was counted after seedling emergence, and soybean root samples were collected two weeks after herbicide application. Based on the data from two locations (Urbana, IL and Monmouth, IL), plant stand was significantly increased in the fungicide treated plots compared to non-treated plots. Analysis of variance revealed significant treatment effects on root rot severity, and lactofen treated plants showed the highest disease severity levels and reduced yields. The azoxystrobin seed treatment provided the best protection against Rhizoctonia root rot disease. There was no significant difference of root rot severity between glyphosate treated and non-treated plants.

Identification of the pathogens caused greenhouse strawberry root and crown diseases in Beijing area, China

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Phytopathology 101:S203

There has been a particularly increasing number of strawberry cultivation in greenhouse in Beijing suburbs in recent years. However, root and crown diseases causing wilt and even death of strawberry have become a serious problem which affects the strawberry production significantly. Pathogen identification was investigated prior to the efficient control of the diseases in strawberry. Sampling was carried out from diseased root and crown of strawberry in greenhouses from five different locations in Beijing during Oct. 2010 to 2011. Pathogen was isolated with regular tissue isolating method and the sequence of the two yellow leaf curl China virus (TYCCNV) were found in Yunnan. Morphologically characterization indicated that the isolates belonged to three respective genera: Colletotrichum, Fusarium and Cylindrocarpon. Reappearance of the symptoms on healthy strawberry plants 3–4 weeks after inoculation with the conidial suspension to the crown also confirmed the pathogen identification. Phylogenetic characterization of tRNA ITS was performed in the isolate of Colletotrichum, demonstrating that the sequence of amplified PCR product with primers ITS1/ITS4 shared a 99% identity with that of Colletotrichum gloeosporioides in GenBank. Herein, one of the pathogens that cause crown disease of greenhouse strawberry from the collected isolates was identified as C. gloeosporioides. As such, species identification of other isolates is undergoing.

Causes of genetic diversities of plant viruses in Yunnan

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Phytopathology 101:S203

There exist abundant resources of plant virus in Yunnan. So far, 13 families, 18 genus and 65 species of plant virus have been found, which account for 50% of the plant virus species of China. The genus Begomovirus has 20 species, Toopovirus has 6 species, Poryvirus has 12 species. 26 isolates of Tomato yellow leaf curl China virus (TYCCNV) were found in Yunnan. Sequence analysis of TYCCNV DNA-A showed all these isolates have the identities of 88.7%–97.4%. There are four causes of genetic diversity of plant virus in Yunnan. Firstly, the geoeological diversity, seven typical climate regions exist from tropical climate to high plateau cold climate. Secondly, the host plant biodiversity, 17000 plant species of Yunnan, account for 62.9% of the total plant species of China, distribute at the diverse climate regions. The rich plant resources are good host plants of many different plant viruses. Thirdly, the population and diversity of the virus-transmitting vector insects such as aphid, whitefly, thrips, leaf hopper are abundant due to the favorable habitats. Forthly, new plant virus isolates or strains are emerging based on the mutation, recombination and reassortment of plant virus. The rich genetic diversity of plant virus in Yunnan provides good natural models for research of plant virus origination and evolution, interaction among plant virus and host plants, virus-transmitting insects.

Development and dispersal of chasmotheca of Erysiphe necator and Podosphaera clandestina, causal agents of powdery mildews of wine grape and cherry

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Phytopathology 101:S203

In eastern Washington chasmotheca are the only known form of perrenniation for the powdery mildew of grape (Vitis vinifera L.) and cherry (Prunus avium L.), caused by Erysiphe necator and Podosphaera clandestina respectively. A 2 yr study was initiated to determine the temporal formation of chasmotheca on infected foliage as well as primary and seasonal modes of dispersal. Chasmotheca development on leaves was tracked from mid-Jul through mid-Oct. Chasmotheca numbers were first formed shortly after harvest in May and late Aug or mid-Sep. Chasmotheca were first formed shortly after harvest in May and late Aug or mid-Sep. Chasmotheca numbers in both pathogens peaked near leaf fall in Oct. Wooden posts 3 m in height equipped with glass slides (oriented vertical and horizontal) coated with silicon grease and filter paper cones were positioned around the periphery of the vineyard and orchard to study dispersal of chasmotheca by air currents. Ascospore viability was assessed using the methods of Cortesi et al. (1997). Peak dispersal for both types of chasmotheca occurred in Oct and was significantly correlated with precipitation and wind speed. The viability of ascospores ranged from 38% to 92% for E. necator and 56% to 96% for P. clandestina. Chasmotheca appear to be loosened by precipitation and then dispersed by rain splash or air currents. In eastern Washington, an area characterized by sustained periods of high winds, dispersal by the latter mechanism may be epidemiologically significant.

Aapoptosis of insect cells S9 and Spev-XII leaded by cantharidin

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Phytopathology 101:S203

To investigate the toxic mechanism of cantharidin (C10H12O4, CTD) against Leptodactyla insubrica, we detected the morphological changes of cell livability and cell cycles arrest of insect cell lines S9 and Spev-XII were observed after treatment by CTD in vitro by means of transmission electron microscope (TEM) and flow cytometry. The results showed that the two lines presented apoptosis features treated with CTD as chromatin condensation, nucleic fragmentation and apoptotic body formation etc. Typical characteristics of cell apoptosis were observed in S9 and Spev-XII cells treated with 3.13 &mu;g/ml and 6.25 &mu;g/ml and in Spev-XII cells with 25 &mu;g/ml of CTD. Flow cytometry detection showed that cells proliferation and growth were inhibited after CTD treatments with the dosage ≥12.5 &mu;g/ml on S9 cells, and ≥2.5 &mu;g/ml on Spev-XII cells respectively. Under the condition of 12.5 &mu;g/ml CTD for 48h, majority of S9 cells showed as cells cycle arrest at G0/G1 phase. Majority of cells treated by CTD exist at the early apoptosis. As the treated concentration of CTD increased the ratio of necrotic cells increased.

Survival of Cercospora sojina on soybean leaves in Illinois

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Phytopathology 101:S203

Historically, frogeye leaf spot (FLS; caused by Cercospora sojina) of soybean has been observed more frequently in the southern U.S. than the North Central U.S. However, in recent years, FLS field observations have been on the increase in the North Central U.S., including Illinois. To better understand the survival ability of C. sojina in Illinois, a field study was conducted across three locations: Monmouth (northern Illinois), Urbana (central Illinois), and Dixon Springs (southern Illinois). At each location, soybean leaves affected by FLS were placed at depths of 0, 10, and 20 cm and retrieved after 12, 19, and 24 months. To determine the viability of C. sojina declined with time equally at all three locations through 19 months. After 24 months, C.
sojina from leaves collected from Monmouth and Urbana was no longer active, but was still active in leaves collected from Dixon Springs. Depth of leaf placement had no effect on survival of C. sojina. These results suggest that planting a non-host crop for two years in central and northern Illinois will reduce the level of C. sojina inoculum to a negligible amount, but non-host crops may need to be planted for a longer duration in southern Illinois to achieve the same effect.

**Isolation, purification and identification of the antifungal protein produced by a newly isolated Bacillus subtilis strain**

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Phytopathology 101:S204

Bacillus subtilis is able to produce many antagonistic substances against plant pathogens. A wild type bacterium producing antifungal protein has been isolated and identified as *B. subtilis*. The *B. subtilis* strain which was named F3 exhibited good growth inhibition against *Monilia fructicola*, so did the culture filtrate of it. The antifungal substance of F3 was crudely prepared by ammonium sulphate precipitation of the culture filtrate. Farther purification was developed by chromatographic separation with Sephadex G-50, TEAE-Sephadex A-25 anionexchange and Sephadex G-100. The first eluting peak of Sephadex G-100 chromatographic displayed obviously antifungal active against *M. fructicola* and showed one protein band in SDS-PAGE. The active chromatographic component was analyzed with MALDI-TOF/TOF mass spectrum. NCBI protein database was searched by Mascot searching tool with P < 0.05 for *Bacillus subtilis*. Flagella contribute to the virulence of pathogenic bacteria and flagellin gene is a good biomark for bacterial detection. The antifungal protein of *B. subtilis* strain F3 was identified as flagellin may indicate that flagellin was a new kind of *B. subtilis* antifungal protein. Thanks to funding project for academic human resources development in institutions of higher learning under the jurisdiction of Beijing municipality (PHR201107135).

**Development and application of a TaqMan real-time PCR assay for rapid detection of Magnaporthe poae**

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Phytopathology 101:S204

Turfgrasses are ubiquitous in urban landscape, athletic fields, golf courses, and residential lawns. Approximately 1.9% (16.3 million hectares) of the total available area is turf. In China, there are more than 195 million hectares of natural forest land. In China, more than 170 species of conifer-infesting bark beetles are known. During the course of the past eight years, surveys of ophiostomatoid fungi associated with economically important bark beetles such as Tomicus spp. and Ips spp. have been conducted in Southwestern and Northern-Eastern China. A total of 750 isolates of Ophiostoma, Grosmannia, Leptographium, Pesotum and Graphium have been identified. They have been identified based on morphological characteristics and these identifications are being confirmed using DNA sequence comparisons. Species such as Ophiostoma abietinum, O. ips, O. piceae, O. quercus, O. setosum, Pesotum fragrans, Leptographium pineti, Grosmannia yunnanensis, Graphium pseudomoricum and eight newly described Leptographium species were characterized and described. A further four Ophiostoma spp. and one Pesotum sp. appear to represent novel taxa, and further study will be needed. The results of this study have greatly expanded our current knowledge of the ophiostomatoid fungi occurring in China.

**Field disease reaction of rice cultivars and elite lines in Texas**

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Phytopathology 101:S204

The development and use of improved disease resistance rice cultivars remains of foremost importance to rice producers. Field evaluation of disease resistance under local environments is essential toward this effort. More than 48 cultivars and Texas elite breeding lines were evaluated for resistance to sheath blight (*Rhizoctonia solani*), bacterial panicle blight (*Buchholzella glumae*) and narrow brown leaf spot (*Cercospora sojina*), the three major rice diseases in Texas, in five trials located at two locations of Texas in 2009 and 2010. Sheath blight was introduced by inoculation while narrow brown leaf spot came from natural infection. Bacterial panicle blight developed from either natural infection or bacterial inoculum at the heading stage. All but several cultivars and lines were susceptible or very susceptible to sheath blight with disease severity ratings of 3 or above on a 0–9 scale. CL142-AR, Jasmine 85, Milagro Filipino, Templeton, and the hybrid XL723 showed partial resistance to sheath blight. In reaction to bacterial panicle blight, all cultivars and lines were susceptible except Catahoula, Jupiter, Spring, XL723, and the two TX elite lines, RU0703190 and RU0703144, which showed partial resistance. Except CL181-AR, Jazzman, Sabine and Sierra, all others were resistant or moderately resistant to narrow brown leaf spot. CL151, Presidio, Tesanai 2, and RU0703190 had grain yields that ranked among the highest.

**Field evaluation of a beneficial Bacillus strain for biocontrol of sheath blight in rice**


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Phytopathology 101:S204

Sheath blight caused by *Rhizoctonia solani* is the most important rice disease in the southern United States. Rice farmers heavily depend on fungicides for control of sheath blight. A field experiment was conducted at two locations to evaluate the efficacy of *Bacillus subtilis* strain MBI-600 and its combined use with a reduced rate of azoxystrobin for management of sheath blight. MBI-600 is the active ingredient in the biopesticide, Integral, and was among the most effective bactericidal strains that were screened against sheath blight in our previous in vitro and greenhouse evaluations. *R. solani* inoculum was introduced into plots at panicle differentiation. Foliar applications of MBI-600 were made at the boot stage. MBI-600, applied to both seed and the foliage at 10^6 CFU/ml, resulted in a significant reduction in sheath blight severity over the untreated control. The combined use of MBI-600 with azoxystrobin at 0.08 kg a.i./ha further reduced disease severity. The efficacy of this combined treatment was comparable to that of azoxystrobin at 0.16 kg a.i./ha. The combined treatment tended to have numerically higher grain yield than the untreated control and have similar yield to azoxystrobin at 0.16 kg a.i./ha. The combined use of the beneficial *Bacillus* strain with a rate-reduced fungicide may provide a practical means to minimize yield losses caused by sheath blight while reducing the usage of fungicides on rice.

**Suppression of soilborne diseases in watermelon and rice with brassica biofumigation crops**

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Phytopathology 101:S204

Fusarium wilt causal induced by *Fusarium oxysporum f. sp. niveum* (FON) and sheath blight caused by *Rhizoctonia solani* are, respectively, the most important soilborne diseases in watermelon and rice worldwide. Chemical control of these diseases is effective. However, chemical control is also costly and may have negative environmental consequences. A research program was initiated.
to develop a biofumigation approach as an alternative for management of these diseases. In vitro assays were performed on agar plates to evaluate the effects of volatile activity of the macerated tissues of *Brassica* and other crops on the growth of *FON* and *R. solani*. Of 37 crops evaluated, 15 *Brassica* crops significantly inhibited *FON*, 17 inhibited *R. solani*, and seven of them, including the mustards ‘Brand 199’, ‘Florida Broadleaf’ and ‘Sheali Hong’, reduced the growth of both pathogens up to 50%. When amended into potted soil in the greenhouse, 12 *Brassica* crops reduced Fusarium wilt incidence by 24 to 72% and increased plant weight by 23 to 183%. Mustard ‘Brand 199’, ‘Florida Broadleaf’ and ‘Sheali Hong’ were most effective in reducing wilt incidence (up to 60%) compared to the nonamended control in microplot and field trials. Use of *brassica* crops may offer a new alternative management for Fusarium wilt in watermelon and sheath blight in rice.

Severe outbreak of bacterial panicle blight across Texas Rice Belt in 2010


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Phytopathology 101:S205

Bacterial panicle blight symptoms have been observed in rice fields in Texas for many years but it was not until 1996 that *Burkholderia glumae* was identified as the causal agent. Although it is generally considered a minor disease, there have been years when significant losses to yield and milling quality occurred. In 2010, a severe outbreak of this disease occurred throughout the Texas Rice Belt. The disease caused partially filled or aborted grains, resulting in an estimated 10 to 20% yield loss and reduced milling quality. The disease was most severe in the cultivars CL111, CL261, Cocomie, and Francis but was relatively less severe in CL151, Jupiter, Neptune, Predisio, and the hybrid XL723. The disease also was present in the ratoon (second) crop but caused no serious damage. *B. glumae* was consistently isolated from the symptomatic panicle samples collected from across the Texas Rice Belt. Of 47 isolates of *B. glumae* collected, 10 were selected for pathogenicity assays and all were shown to be pathogenic. Two of the pathogenic isolates, one from the main crop and the other from the ratoon crop, were further verified to be *B. glumae* using PCR. This is the first report of bacterial panicle blight of rice in the ratoon crop in the United States resulting from an epidemic outbreak of this disease in Texas. This disease poses a threat to rice production since there are no chemicals or highly resistant cultivars currently available for management of this disease.

Molecular characterization of a Chinese isolate of *Chickpea chlorotic stunt virus* infecting pea

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Phytopathology 101:S205

Chickpea chlorotic stunt virus (CpCSV) causes yellowing and stunting of *P. vulgaris* seedling stage, separately. The results showed that the identification effect was uniform in two methods. It indicated the reliability and precision of tiller inoculation. The breeding process and genetic research for rice stripe disease resistance should be accelerated by using this method in the early generation selection.

Pathogenicity analysis of secretory protein of the rice blast fungus and interaction study using rice cell suspension culture

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Phytopathology 101:S205

The rice blast fungal strains CH-63 and TH-16 with 2 different pathogenic factors were cultivated in a liquid culture medium under nitrogen starvation. After 2 d of cultivation, the protein secreted by the fungal strains was extracted by ammonium sulfate sedimentation and purified by dialysis. A rice plant was infected with the secreted protein of the rice blast fungal strains through inoculation or soaking method. A necrotic lesion was observed in the rice leaf and stem at the inoculated position; the lesion diameter was 2 to 4 times that of control. Soaking resulted in severe browning of the rice radicle, and the inoculated plant height was only half of that of control. In the rice plant inoculated with the secreted protein, the rice disease symptoms disappeared or weakened after treatment with protease K. This finding further confirmed that the secreted protein was the major pathogenicity factor.

Twenty-four monogenic rice lines developed at International Rice Research Institute (IRRI) were inoculated with the secreted protein and then categorized into resistant, intermediate resistant, and susceptible groups based on the size of necrotic lesion observed on the rice leaf tissue after 72 h of inoculation. The results showed that the CH-63 strain showed higher pathogenicity than the TH-16 strain. The difference between the pathogenicity of these 2 strains was proved by trypan blue staining.

Molecular mapping of new genes for stripe rust resistance in spring wheat genotypes PI 178759 and PI 183527

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Phytopathology 101:S205

New genes are essential for breeding wheat cultivars with effective resistance against stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*). Spring wheat PI 178759 and PI 183527 were identified to have high-levels of resistance in fields in 2004 to 2010. Further testing under controlled greenhouse conditions with individual races showed that PI 183527 had a typical high-temperature adult-plant (HTAP) resistance while the HTAP resistance in PI 178759 was more temperature-sensitive. The genotypes were crossed with susceptible spring wheat Avocet. Genetic analysis identified two genes in PI 178759 and one gene in PI 183527 using the phenotypic data of *F2* and *F3*. Each with individual races of *Pst* tested in the field under natural infection of the pathogen. Molecular mapping using resistance gene analog polymorphism and simple sequence repeat (SSR) markers located the three genes on the long arm of chromosome 7B. The two genes in PI 178759 were 39.1 cm away. One of the PI 178759 genes was linked in repulsion with the PI 183527 gene. Both genes were flanked by SSR markers *Xbarc182* and *Xcfe2040*. Allelism testing is the best way to determine if the two genes are at different loci. Based on the evidence, the two genes in PI 178759 and PI 183527 appeared to be different from previously identified genes and should be useful for developing wheat cultivars with durable resistance to stripe rust.

Metagenomic analysis of *Candidatus Liberibacter asiaticus* in naturally populated psyllids (*Diaphorina citri*) using BAC libraries

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Phytopathology 101:S205

*Candidatus Liberibacter asiaticus* (Las) is the most prevalent species of three species of Ca. Liberibacter causing citrus huanglongbing (HLB) in the world. The Las gene sequence published in 2009 was obtained from a single Las isolate using metagenomic approach. Studies based on the diversity suggest a strong potential of various populations of Las bacteria exiting in different hosts. In this study, three BAC libraries were constructed using whole genomic DNA from thousands of *Diaphorina citri* collected from the HLB-affected citrus plants in the fields. A total of 61,440 clones were obtained from three libraries constructed by partial digests of *BamHI* and *HindIII*, respectively, or random shear. Superpools and pools of DNA from the *BamHI* BAC library were screened by conventional PCR using Las-specific primers. Thirty sets of the Las-specific primers were designed with a
distance from 30-50 kb based on the Las-psy62 genome. Positive BAC clones were identified and subjected to end sequencing. The results indicated 54 overlapping clones that matched the Las genome sequence with a size range from 20 kb to 140 kb. PCR confirmation of the end sequence using infected vs. non-infected plants or insects with the primer sets designed from the end sequences revealed 7 overlapping clones contained new sequences that were missed in the psy62 genome, while 15 clones did not match the Las sequence, suggesting a potential chimera in these BAC clones.

Sweet bundle of sugarberry - a novel ampelovirus found in Celtis laevigata

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Phytopathology 101:S206

Virus-like symptoms are observed in several Celtis species across the Southern United States. The most striking symptoms of viruses are seen on sugarberry (Celtis laevigata) where bright yellow mottling appears in late spring and becomes more prominent as the season progresses. Here we report a new virus, closely associated with the bright mottling symptoms. The new virus has a monopartite, single-stranded RNA genome consisting of approximately 17 kb. Protein pairwise comparisons and phylogenetic analysis show that the virus is most closely related to Grapevine leafroll-associated virus-3, the type member of the genus Ampelovirus, family Closteroviridae. The amino acid identities between the two viruses range from 53% for the RNA dependent RNA polymerase to 26% for the coat protein homolog signifying that the new sugarberry virus is a novel member of the genus. Detection protocols have been developed and the virus was detected in several other Celtis species showing yellow mottling symptoms. High numbers of mealybugs, known vectors of ampeloviruses, are often seen on affected trees and transmission studies are underway to determine whether they can transmit the virus.

Fusarium verticillioides infection of maize seedlings and the corresponding movement of fungus, fumonisins, and biomarkers of exposure

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Phytopathology 101:S206

In earlier studies using maize seedlings grown from kernels inoculated with Fusarium verticillioides, fumonisin B1 (FB1) was preferentially accumulated in leaf tissue compared to FB2 and FB3, whereas, in plants watered with purified toxins there was no accumulation of fumonisins in the leaves. The present study was designed to validate the effects observed previously, but utilized FB producing and non-producing strains of F. verticillioides. This study correlated fungal infection with accumulation of FB1, FB2 and FB3 and sphingolipid biomarkers in plant tissues. Different maize lines were used to determine their resistance to FB and sphingolipid biomarker accumulation compared to the susceptible lines. The maize lines showed fewer effects from pathogenesis and less FB1 and biomarker accumulation compared to the susceptible lines.

The ShyA/MarR family regulator Hor regulates HrpL regulon T3SS genes in a HrpL dependent manner

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Phytopathology 101:S206

The hpr genes of Dickeya dadantii 3937 encode a type III secretion system (T3SS) which is essential for its full virulence. Previous studies of the T3SS regulation in D. dadantii revealed that the expression of the hpr genes is regulated by HrpL, an alternative sigma factor, through the HrpX-HrpY- HrpS-HrpL and GacS-GacA-rsmB-RsmA pathways. In this work, we identified a novel T3SS regulator of the MarR/SlyA family, Hor, which regulates hpr regulon genes through a HrpL independent pathway. We demonstrated that the expression of hprL was enhanced in a hor deletion mutant, due to an enhanced hprS expression and a reduced rsmA expression. However, the expression of hprA and hprN, two hpr genes in the HrpL regulon, was greatly reduced in the hor mutant. Interestingly, concomitant with its up-regulation of the T3SS, Hor exerts a negative regulatory effect on the production of extracellular enzymes such as pectate lyase (Pell), cellulase (Cel), and protease (Prt) in D. dadantii. These results indicate that Hor plays a role in coordinate regulation between two virulence factors, T3SS and extracellular enzyme production. Finally, we demonstrate that Hor also controls bacterial swimming motility, pellicle formation, and bacterial virulence in D. dadantii.

Characterization of the host defense response induced by the flagellin protein of Candidatus Liberibacter asiaticus

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Phytopathology 101:S206

The pathogen-associated molecular patterns (PAMPs) are recognized by plant receptors leading to PAMPs-triggered immunity. The highly conserved N-terminus domain of flagellin (Flg22) is an extracellular PAMP that is recognized by most plant species. In the genome of Candidatus Liberibacter asiaticus’, the causal agent of citrus Huanglongbing, there is only one copy of flagellin-encoding gene in contrast to four copies of them in Sinorhizobium meliloti. Transient expression of the Las-flagellin using PVX expression vector induces an immunity response with cell death in Nicotiana benthamiana. The flagellin consists of 22 amino acids near N-terminus (-DRVSSGLRVSD AADNAAYWSIA-), sharing the conserved Flg22 domain. By comparison with the nonfunctional homologue from S. meliloti, three divergent amino acids were identified. To test if this PAMP exists in citrus, 10 µM commercial Flg22 (RP19986, GenScript) and Flg22-Las were infiltrated into young tissue of citrus leaves, respectively. The results indicated these peptides induced hypersensitive response in young citrus leaves. This is first evidence of citrus defense response to the phloem-limited ‘Ca. L. asiaticus’.

S206 PHYTOPATHOLOGY
The role of copper in rice–Xanthomonas oryzae interaction
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Phytopathology 101:S207

Copper is an essential micronutrient of plants. It is also an important element in a number of pesticides. Chemical control of rice bacterial blight disease caused by Xanthomonas oryzae pv. oryzae (Xoo) began in the 1950s with the preventive application of Bordeaux mixture, which was the world’s first commercially successful fungicide and bactericide, a simple mixture of CuSO4 and hydrated lime. Rice Xa13 is a Xoo susceptible gene. Xoo strain PXO99 and copper induces its expression. Xa13 encodes an indispensable plasma membrane protein of the MtN3/saliva family. This protein cooperates with two other proteins, COPT1 and COPT5, to promote removal of copper from xylem vessels, where Xoo multiplies and spreads to cause disease, to the cells around the vessels. Utilization of Xa13 by the bacterium to facilitate its infection presents a novel molecular mechanism of coevolution between hosts and pathogens.

Arabidopsis-Pseudomonas syringae interaction provides insight into PAMP-triggered immunity
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Phytopathology 101:S207

Plants are equipped to sense pathogen/microbe-associated molecular patterns (PAMPs/MAMPs), which are conserved molecular signatures among microbes. The perception of PAMPs by plant cell surface-localized pattern recognition receptors (PRRs) rapidly activates mitogen-activated protein kinases in Arabidopsis. These MPKs are thought to represent two distinct MAPK cascades regulating plant immunity. The signal transduction mechanisms underlying PAMP-triggered immunity, however, are poorly understood. The bacterial pathogen Pseudomonas syringae secretes a large repertoire of effector proteins into the plant cell to enhance virulence. We have shown that many of these effector proteins inhibit PAMP-triggered immunity by targeting key signaling components. We are using these effector proteins as molecular probes to dissect plant immune signaling pathway. For example, the analysis of host targets for the effector AvrPphB has led to the identification of BIK1 and PBL protein kinases as new components of PAMP-signaling pathway. As an alternative approach, we are conducting genetic screens to uncover additional components. We will present our results and discuss the advantage and limitations of each approach.

Abstracts of Special Session Presentations

Hot Topic

Current Advances of Molecular Plant Pathology in China

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Plant defense and geminivirus counter-defense
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Phytopathology 101:S207

RNA silencing is a natural defense mechanism which acts as an adaptive immune system against invading nucleic acids such as viruses in plants. Geminiviruses, contains a circular, single-stranded DNA genome and replication occurs in the nucleus by a rolling circle mechanism that employs circular double-stranded DNA, are thought to be both inducers and targets of RNA silencing. To counteract an antiviral RNA silencing response, geminiviruses express silencing suppressor proteins. Tomato yellow leaf curl China virus (TYLCCNV), a geminivirus, was found to be associated with betasatellite (TYLCCNB). Methylated TYLCCNV DNA was detected in TYLCCNV-infected Nicotiana benthamiana plants, but TYLCCNB significantly reduced the cytosine methylation level of TYLCCNV. The βC1 encoded by TYLCCNB was found to function as a suppressor of transcriptional gene silencing (TGS). Over-expression of βC1 from a Potato virus X (PVX) vector reversed TGS of a GFP transgene and caused genome-wide reduction of cytosine methylation in infected N. benthamiana plants. Taken together, we demonstrated that DNA methylation is an important plant defense against TYLCCNV, and as a counterdefense, βC1 encoded by TYLCCNB could reverse TGS. The mechanisms of suppression are being progressively unraveled.

Update on interactions between wheat and stripe rust pathogens
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Phytopathology 101:S207

Stripe rust, caused by Puccinia striiformis (Pst), is a serious disease in wheat worldwide. The use of resistance cultivars offers the most economic and environmentally friendly way to control the disease. To better understand mechanisms of wheat-Pst interactions, researches on transcriptional analysis of wheat-Pst interactions using RNA and microRNA sequence techniques were conducted. RNA for solexa sequencing were extracted from Pst-infected and mock-treated wheat leaves at 24, 48 and 120hpi. For transcriptome, over 150000 unigenes, ranging in size from 200bp to 6000bp, were obtained, and protein functional annotation was performed with a local EST analysis platform. Based on comparative analysis of transcripts, 2564, 910 and 494 differentially expressed unigenes (DEGs) in wheat attacked by virulent Pst race at 24 48 120hpi were isolated, respectively, and 2666, 783 and 2587 DEGs in resistance interaction. Furthermore, some interesting genes were selected for...
functional characterization by VIGS and RNAi. For MicroRNA, A total of 134 differentially expressed MicroRNA were generated in incompatible interaction, whereas 117 in compatible interaction. MicroRNA array and northern blot analyses showed that some microRNAs were up- or down-regulated in wheat-Pst interactions. We uncovered a number of new interesting genes possibly involved in wheat response to Pst infection, which will be helpful to decipher the molecular mechanism of wheat-Pst interactions at different level.

Biology of Pathogens

Fungal Comparative Genomics and the Impact of Next Generation Sequencing

Mycosphaerella comparative genomics reveals chromosome dynamics, genome evolution, and stealth pathogenesis

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Phytopathology 101:S208

Mycosphaerella graminicola causes septoria tritici blotch, one of the most important diseases of wheat worldwide. Previous analyses showed that populations of this species are extremely variable and that polymorphisms for chromosome length and number can be generated during meiosis. To better understand the genetic basis for genomic plasticity, the genomes of M. graminicola and the related banana pathogen M. fijiensis were sequenced by the Joint Genome Institute. The finished genome of M. graminicola had 13 core chromosomes and a dispensable of eight chromosomes that were different from those in the core for every parameter measured. Comparison with sequences of related species revealed that the dispensable probably originated by ancient horizontal transfer from an unknown donor, and has been maintained through at least one speciation event. Content (genes and transposons) on the core set is conserved among species and 13 seems to be the basic chromosome number in Mycosphaerella. Content on the eight dispensable chromosomes is conserved within populations but not within individuals, and some parts of the dispensable occur at lower frequency than others. Species of Mycosphaerella contain fewer genes for wall-degrading enzymes than pathogenic species of otherrust fungi and may metabolize proteins rather than carbohydrates during the biotrophic phase of growth. Stealth pathogenicity may have evolved to avoid detection by the host, possibly from an endophytic ancestor.

Verticillium comparative genomics yields insights into niche adaptation by plant vascular wilt pathogens


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The vascular wilt fungi Verticillium dahliae and V. albo-astrum infect over 200 plant species, causing billions of dollars in annual losses. The characteristic vascular wilt symptoms are a result of colonization and proliferation of the pathogens in the xylem vessels. To gain insights into the mechanisms that confer pathogenicity among wilt fungi, we sequenced two Verticillium wilt pathogens and compared their sequences to each other, and with the proteome of Fusicoccum oxysporum, another fungal wilt pathogen. Among a set of proteins conserved in the three wilt fungi, we identified homologs of a bacterial virulence factor that was likely acquired by the fungi through horizontal transfer events, and may contribute to the adaptation to proliferate within the plant xylem. Compared to other fungi, the Verticillium genomes encode more plant cell wall degrading enzymes, providing an extraordinary capacity to degrade plant pectin. Comparison of the two closely related Verticillium genomes uncovered variable genomic islands in the primary causal agent of Verticillium wilts, Verticillium dahliae. Coupled with the impressive arsenal of plant cell wall-degrading enzymes, the variable genomic islands may provide enhanced genetic diversity for host range expansion. In summary, our study reveals insights into niche adaptation of fungal wilt pathogens and sheds light on avenues to follow for the development of disease management strategies.

Genome dynamics of the Fusarium oxysporum species complex

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Phytopathology 101:S208

The Fusarium comparative genomes of F. graminearum, F. verticillioides and F. oxysporum revealed greatly expanded lineage-specific (LS) chromosomes in F. oxysporum. These LS chromosomes contribute to the organism pathogenicity and host-specificity, providing explanation for the polyploidy origin of host specificity and the emergence of new pathogenic lineages in the F. oxysporum species complex (FOSC). Following this discovery, a comparative study focusing on the members of FOSC was developed to: 1) study genome structural variation and to confirm the presence of the LS chromosomes among different isolates using optical mapping technology; 2) study gene content variation among these selected isolates using next-generation sequencing (NGS) technology; 3) obtain genes encoded in the LS chromosomes. One human isolate and 11 plant pathogenic isolates that represent 8 forma specialis were included in the study. Our study confirms the genome dynamics of the FOSC. The result from the optical mapping process confirms the existence of LS chromosomes in all different isolates we investigated using this technology. The genomic data generated using NGS reveals the power in detecting genome-wide mutation pattern in short evolutionary divergent time, while RNA-seq data shows great promise in detecting novel genes encoded in the LS chromosomes and studying the gene expression under different conditions.

New insights into the obligate biotrophic lifestyle of rust fungi through comparative genomics


Phytopathology 101:S208

Wheat production continues to be plagued by rust pathogens and with the recent race shifts there is an increased concern for wheat global food security. Three distinct rust fungi caused disease in wheat: Puccinia graminis f. sp. tritici (Pgt), stem rust or black stem rust; P. striiformis f. sp. tritici (Pt), stripe rust or yellow rust; P. tritici (Pt), leaf rust or brown rust. These three rust fungi have complex life cycles and the asexual dikaryotic uredinial stage is the only practical form for genome sequence analysis and assembly. Genomes of these rust fungi are large (~127 Mb) and complex with a high level of repetitive sequences (approximately 45%) primarily due to massive proliferation of transposable elements. These genomes are highly polymorphic within and between isolates; Pgt contains ~ 1 SNP/kb between haploid genomes within an uredinial spore. Of the 18,240 predicted proteins in the Pgt genome, approximately 1,000 are small-secreted proteins (SSPs) and may be involved in pathogenicity. Gene expression analysis using microarray and RNA-sequencing identified a subset of the SSPs that are highly upregulated in plants and/or isolated haustoria. In addition, many of these SSPs are differentially expressed between races of Pgt. Transient expression analysis is being used to determine the function of a selected set of SSPs. Comparative analysis of these three wheat rust genomes (Pgt, Pt and Pp) will be discussed.
Discovery of new soybean and soybean rust genes using next generation sequencing
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Phytopathology 101:S209
Soybean is one of the top five agricultural products in the United States and is highly susceptible to soybean rust (SR), an exotic obligate fungus. We used mRNA-Seq by Illumina to analyze gene expression patterns of the host and pathogen at different time points during infection of the leaf and we also discover new genes. We found sequences aligning to the soybean genome where no gene is annotated. A good example is a contig aligning between two genes on chromosome 6. One translates, one open reading frame encoding five proteins was revealed between these genes. Four proteins had high similarity to retrotransposon proteins from plant while one protein had high similarity to a disease resistance protein from Brassica rapa. Many other contigs did not align to the soybean genome and did not have homology to SR sequences. Sequence information on SR is limited; therefore many of these unknown sequences may be from SR. Some of these unknown contigs had similarity with genes in different public databases encoding proteins involved in fungal development, lignin degradation, signal transduction and intracellular communication while in some others only conserved domains such as signal peptides, common to fungal virulence factors, catalase and peroxidase, common to proteins involved in defense, could be identified. Such information may be use to update our knowledge of the soybean and SR genomes and to develop new methods to broaden resistance of soybean to soybean rust.

Diseases of Plants

Disease Complex Between Nematodes and Other Plant Pathogens
The nightmare of plant diseases associated with soybean cyst nematodes
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Phytopathology 101:S209
Soybean cyst nematode (SCN), Heterodera glycines, can directly reduce soybean yields by 30% in the absence of symptoms, and yield losses up to 70% have been described. Even though SCN is an introduced species in North America, the “single causation principle” probably does not apply for most losses attributed to SCN. The nematode can indirectly affect soybean yields through interactions with various mutualistic and parasitic symbiotes of soybean in ways described as additive, synergistic, or antagonistic. The interacting organisms include, among others: Bradyrhizobium japonicum, Calonectria crotalariae, the pathogenic fungus Fusarium oxysporum f. sp. dianthi, the bacterium; Pseudomonas fluorescens strain GcM5-1, carried by PWN. These interactions indicate a symbiotic relationship between PWN and the bacterial strains. Ten bacterial strains and five nematode species were spatially separated from the fungus, demonstrating the importance of P. penetrans in PED for two potato fields by manipulating nematode and fungus inoculum densities using cover crops and solarization. Mean soil inoculum densities were negatively related to yield for P. penetrans x V. dahliae. Elucidating the Verticillium wilt disease and host resistance to it may not reveal an alternative to soil fumigation for the potato industry. Surveys of potato fields in Wisconsin, Maine, Canada, and Australia showed most fields were infested with Pratylenchus spp. as well as V. dahliae. We studied the importance of P. penetrans in PED for two potato fields by manipulating nematode and fungus inoculum densities using cover crops and solarization. Mean soil inoculum densities were negatively related to yield for V. dahliae for one experiment, and for P. penetrans and the P. penetrans x V. dahliae interaction in both experiments. Evidence supports the value of including nematodes in PED research and the need to better understand the pathogenicity of Pratylenchus spp. to potato.

Is it nematode or fungus that causes Mr. Potato to die early?
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Phytopathology 101:S209
The potato early dying disease (PED) is caused by an interaction of Verticillium dahliae with other pathogens, particularly Pratylenchus spp. This common disease reduces the yield and quality of most potato cultivars. Controlled inoculation showed a synergistic increase in PED symptoms when nematodes were spatially separated from the fungus, demonstrating the interaction is not simply root wounding. The collaboration of fungi and nematode for PED, verified in a wide range of environments, is accepted by farmers and successfully managed using soil fumigation. A majority of recent studies to understand the molecular and genetic basis of PED have focused exclusively on V. dahliae. Elucidating the Verticillium wilt disease and host resistance to it may not reveal an alternative to soil fumigation for the potato industry. Surveys of potato fields in Wisconsin, Maine, Canada, and Australia showed most fields were infested with Pratylenchus spp. as well as V. dahliae. We studied the importance of P. penetrans in PED for potato fields by manipulating nematode and fungus inoculum densities using cover crops and solarization. Mean soil inoculum densities were negatively related to yield for V. dahliae for one experiment, and for P. penetrans and the P. penetrans x V. dahliae interaction in both experiments. Evidence supports the value of including nematodes in PED research and the need to better understand the pathogenicity of Pratylenchus spp. to potato.

Pine wilt disease: From nematology to quarantine
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Phytopathology 101:S209
Bursaphelenchus xylophilus (PWN) has been thought to be the sole pathogen causing pine wilt disease, even though the disease mechanism has not been clearly elucidated. Based on a series of experiments, we hypothesized that pine wilt disease is a complex disease induced by PWN and bacteria it carries. Bacterial strains in the genus Pseudomonas were isolated from PWN in diseased pines (Pinus thunbergii) in China. The bacteria were bioassay on axenically grown PWN on callus of Pinus thunbergii. Ten bacterial strains and PWN supporting each other in their development and reproduction, that indicates a symbiotic relationship between PWN and the bacterial strains. Flagellin, a lignin peroxidase and two cyclic dipeptides were isolated from the bacterium; Pseudomonas fluorescens strain GcM5-1, carried by PWN. These compounds were tested for their toxicity to callus and seedlings of P. thunbergii. Results showed that flagellin could affect the cell membrane of P. thunbergii and increase its permeability, which led to the leakage of cell electrolytes and wilting. At the same time, the toxin treated cell wall was disrupted. This result explained the mechanism of pine wilting and death. This new theory was supported by field tests with a formulation of a nematicide and a bactericide in Korea by Kim J. C. et al. (2010). The current pine control strategies and quarantine rules against PWN will be changed according to these new findings.

Viruses transmitted by nematodes: When the germs meet the worms
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Phytopathology 101:S209
The first report that plant-parasitic nematodes transmit plant viruses was published in 1958. Since this time, 30 plant-parasitic nematode species within the Longidoridae and Trichodoridae have been shown to transmit 14 nepoviruses or tobaviruses. These nematode-virus disease complexes affect a wide range of annual and perennial crops, resulting in economic losses and quarantine restrictions. During the last 54 years, numerous avenues of research have been taken to elucidate the nature of the association between virus and nematode, including: mechanisms of acquisition and transmission of viruses by nematodes, identification and distribution of nematodes and viruses they transmit, pathogen ecology, and disease management. Recent advances in nematode and virus diagnostics, transgenic plant resistance, and nematode-virus interactions at the molecular level will provide further insight into the management of these unique vector-virus complexes in the future. A historical perspective and current research directions on the topic will be discussed with specific examples from disease complexes involving Xiphinema indexi grapevine fanleaf virus, Paratrichodorus spp./tomato rattle virus, and Xiphenima spp./tomato and tobacco ringspot virus.

You think the root-knot nematode is the only culprit?
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Phytopathology 101:S209
Root-knot nematodes have also been implicated in disease complexes with a number of fungi in cotton. The most famous of these, the root-knot nematode–Fusarium wilt complex, has received considerable research attention since its original description shortly before 1900. Other nematode–fungal associations on cotton have also been described as additive or synergistic to characterize...
Management of Insect-Transmitted Plant Virus Diseases in the Tropics

The role of epidemiology in the management of insect-transmitted viruses—A biological perspective

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Phytopathology 101:S210

Epidemics caused by insect-transmitted viruses in cultivated plants pose a worldwide challenge to achieving satisfactory yields and quality of produce. This is especially so in tropical climatic zones where insect vectors are often abundant and epidemics occur in situations ranging from primitive subsistence cropping systems to technologically very advanced ones. An increasingly sophisticated and diverse range of host resistance, phytosanitary, cultural, chemical, biological and legislative control measures are becoming available to meet this challenge. Thorough knowledge of the epidemiology of pathosystem concerned is critical to decision making over control. When combined with sound information on the selectivity, mode of action, effectiveness and reliability of each individual control measure, and how to respond, this epidemiological knowledge is used to decide which control measures to deploy and whether to use an individual measure alone or together with others. However, control measures suitable to recommend for primitive subsistence cropping systems need to be tailored very differently from those suitable for technologically very advanced ones. Also, to be adopted control measures also need to be ecologically and socially sustainable, robust, affordable and compatible with local agricultural practices. Fortunately, there is an ever-increasing knowledge base and sophistication of technology to draw on to help in decision making.

Whitefly and Begomovirus biology as a tool for their management in a developing country: Guatemala

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Phytopathology 101:S210

Research has been conducted to correlate Bemisia tabaci biotypes with the presence of different Begomovirus species in tomato and chile pepper in five Guatemalan regions in order to manage these crops without excessive insecticide usage. Seven B. tabaci biotypes were found using the CO1 gene. The polymorphisms are based on only eight nucleotide substitutions or deletions of the total 780 bp. The biotypes were found to correlate with crop, region, season and altitude. We found Tomato yellow leaf curl virus (TYLCV) present in 2006 in Jutiapa, El Progreso and Baja Verapaz. Further sampling throughout the country showed an incidence of TYLCV of 2.4% in plants and 0.7% in B. tabaci in nine additional departments. Another six Begomovirus species were found, the most abundant being Tomato severe leaf curl virus (ToSLCV), Pepper golden mosaic virus (PepGMV), Tomato golden mosaic virus (ToGMoV) and Tomato mosaic Havana virus (ToMHV), in both plants and whiteflies. This new information will facilitate Begomovirus management by, for example, the use of host free periods in the season when whitefly populations are highest.

Implementation and success of host-free periods for managing tomato-infecting begomoviruses in developing countries

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Phytopathology 101:S210

Plant diseases caused by whitefly-transmitted geminiviruses (genus Begomovirus) cause major losses to vegetable crop production, especially in tropical and subtropical regions of the world. These diseases are difficult to control and result in farmers applying large quantities of insecticides. This practice is generally not effective and is undesirable for many reasons. Thus, effective sustainable practices are needed for disease management. This requires a thorough understanding of the virus(es) involved and the virus-vector interaction. In many regions, investigation of the begomovirus(es) involved and their biological properties have revealed that these diseases are caused by locally adapted viruses with narrow host ranges, often with the susceptible crop plant being the primary host. This has led to development and implementation of regional crop or host-free periods as a management strategy. This practice involves not growing the susceptible crop plant, usually for a period of 2–3 months, thereby disrupting continuous cropping patterns. This can result in a dramatic reduction in virus inoculum in the agroecosystem and, in some cases, a reduction in the insect vector population. Two successful applications of a host-free period for managing tomato-infecting begomoviruses in developing countries with tropical environments will be presented. In addition, the biological, economic and social aspects of developing and implementing a host-free period will be discussed.

Management of Peanut bud necrosis virus disease in tomato in South Asia

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Phytopathology 101:S210

Thrips-transmitted tspoviruses (genus Tospovirus, family Bunyaviridae) are a significant limiting factor in the sustainable production of vegetable, food, feed and fiber crops worldwide. Among them, Peanut bud necrosis virus (PBNV) has emerged as a major constraint to tomato production in South Asia, especially in India. Reliable information on the occurrence of PBNV in other countries is lacking, although its presence was recently confirmed from Indonesia. PBNV is transmitted by Thrips palmi and has a broad host-range including legume and solanaceous crops and non-crop species. Because of limitations and disadvantages of chemical control of thrips, broad host-ranges of PBNV and its vector, overlapping cultivation of susceptible crops, and the lack of genetic sources of resistance to PBNV in tomato, the IPM-CRSP of the USAID is pursuing environmentally benign IPM strategies as an alternative to pesticide-based tactics for mitigating negative impacts of this virus to tomato production. Farmer-participatory appraisals, multi-location evaluations of tomato cultivars and hybrids in combination with roguing of symptomatic tomato seedlings during and soon after transplanting are offering simple but robust technologies to farmers for reducing virus incidence and avoiding crop losses. An analysis of benefit-cost ratio of roguing concluded that farmers can gain additional revenue without incurring extra costs for spraying pesticides to control thrips vectors.

Whitefly vector populations in relation to virus ecology and management

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Phytopathology 101:S210

Initial molecular studies identified two mt-COI haplotypes (UG1, UG2) of the whitefly vector Bemisia tabaci associated with the severe Cassava mosaic disease (CMD) outbreak in Uganda ca.1988-90, which now affects eleven countries in east and central Africa. Phylogenetic analysis of the mt-COI and an informative nuclear marker (KDR intron) revealed that UG1 is endemic to biology and that UG2 represents an exotic, introduced haplotype. Five unlinked, informative microsatellite markers were subsequently isolated from two independent enriched genomic libraries from whiteflies from eastern and western Africa that show clear structure at the desired level, and differentiate between pre- and post-invasion scenarios. Analysis of field collections provided by our collaborator Dr. James Legg, IITA-Tanzania will be presented. Results demonstrate the use of molecular and population data to inform vector biology in relation to spread of the severe CMD pandemic in cassava in sub-Saharan Africa that enable optimal targeting of virus-resistance varieties distributed to the highest risk locales. Such data also provide a baseline for understanding whitefly complexity in relation to their role as vectors of begomoviruses and other emerging viruses in the Caribbean, Central America and Asia.

Challenges unique to managing viruses in tropical developing countries

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Phytopathology 101:S210

Plant viruses transmitted by insect vectors or through seed or germplasm cause diseases that become major constraints to food security in tropical
developing countries. Diseases caused by viruses pose extreme challenges to host country scientists because of limited capability and resources to diagnose and understand the virus/vector/host pathosystem, essential for management within the increasingly dynamic crop ecosystems of developing countries. The lack of capacity and infrastructure to conduct rapid diagnostic assays is being alleviated somewhat by collaborative research and training and by improved diagnostics. As prevalence of the most economically important viruses in an area is documented through surveys, this knowledge will enable targeted research toward understanding the complexity of virus biology in diverse cropping ecosystems and dissemination by vectors (e.g. aphids, thrips and whiteflies). The basic concepts of managing viruses by reducing initial inoculum and delaying infection and rate of spread remain applicable. However, technologies for tropical cropping ecosystems must be holistically designed and transferred to different regions/countries, and have socioeconomic acceptance and impact.

New and Emerging Technologies in Turfgrass Disease Management

The history and new advances in fungicide development for turfgrass disease management

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Phytopathology 101:S211

Turfgrass disease management has been linked with fungicide development since the inception of turfgrass pathology. The initial experiments conducted on turfgrass disease control were performed at the Arlington Turf Gardens in 1917. The objective of these initial trials was to determine the efficacy of Bordeaux mixture against brown patch of fescue. The results of these trials demonstrated the benefits of fungicide applications greatly outweighed the disadvantages. Leaving the era of copper in fungicide history behind, turfgrass disease management entered a new era, one with synthetic organic compounds. Many notable products became available in middle 1900’s for the control of turfgrass diseases such as benomyl, thiophanate methyl, iprodione, chlorothalonil, propiconazole and mancozeb. Although these fungicides were initially highly effective, after a few to many years of use, some of them succumbed to resistant pathogens. Fungicides coming to the market in the 2000’s and presently, typically have single site modes of action that are prone to the development of fungicide resistance. Moreover, the increasing costs of bringing a new pesticide to market and the competition from post-patent products have hindered the release of new compounds. Consequently, this has ushered in a new age of studying the basic biology of turfgrass diseases and the efforts of today’s turfgrass pathologists will likely revolutionize how we manage turfgrass diseases in the future.

Advances in application technology for turfgrass disease management

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Phytopathology 101:S211

Intensively-maintained turfgrass is susceptible to a wide array of fungal diseases. Golf course and putting greens and fairways often receive numerous fungicide applications annually. The efficacy of a fungicide application depends on applying the right products at the right time using the right equipment and the correct technique. In recent years there has been a surge of interest in the influence of application technology for turfgrass disease management, including studies on nozzle selection, water carrier rates, and other factors. Along with improved disease control, drift reduction is another key goal. Many golf course superintendents are innovators who are eager to try new methods. This presentation will summarize recent progress in research and outreach related to application technology for turfgrass diseases including dollar spot (Sclerotinia homoeocarpa), spring dead spot (Ophiostoma spp.), brown patch (Rhizoctonia solani), fairy rings (various causal agents), and others.

Turfgrass diagnostics and new, advanced technologies

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Phytopathology 101:S211

Strategies for sustainable, integrated disease management start with reliable pathogen identification. Conventional identification methods such as disease symptomology, host association, morphology and biochemical tests are still key diagnostic indicators for many phytopathogens; however, nucleic acid recognition-based methods are increasingly used for routine pathogen identification due to their speed, accuracy, sensitivity and reproducibility. In this seminar, we provide an overview of nucleic acid-based recognition tools for turfgrass pathogen identification that are currently available or under development, including real-time PCR, microarray, SNP and microsatellite profiling, DNA sequence analysis and bioinformatics. We also provide examples of successful real-world applications of DNA-based turf pathogen identification tools currently being used by diagnostic laboratories, pathologists and plant breeders, and discuss innovative new technologies that could facilitate the future deployment of molecular identification tools for use in the field or lab without the requirement for sophisticated instrumentation.

Enhancing systemic resistance in turfgrass disease management

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Phytopathology 101:S211

Turfgrasses are known to possess natural defense mechanisms against stresses including diseases. Under intensive maintenance systems, these natural mechanisms may be insufficient to guard the plants against disease outbreaks. There are chemicals that have been observed to stimulate the natural resistance pathways in plants, with more abundant research on broadleaf plants as compared to grasses. We have been investigating the mechanism of action of certain new compounds in their role of defense activation against diseases in plants. These compounds generally do not have strong direct anti-fungal effects, but activate signaling pathways within the plant to either cause direct expression of defense-related genes prior to pathogen attack (induction) or allow expression of defense-related genes more quickly in response to pathogen attack (priming). Using tests in the lab and in the field, we have found that applications of such chemicals either alone or in combination can reduce turfgrass diseases significantly. This presentation will explore the use of such chemicals for turfgrass disease control, and discuss their advantages and possible disadvantages.

Using molecular tools to improve our knowledge of turfgrass pathogens

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Phytopathology 101:S211

Traditionally, turfgrass pathology research has been primarily focused on management practices that were relevant or applicable to the turf industry. Basic investigations into turfgrass pathosystems have been conducted sporadically for years but have not been as common or extensive as long standing applied efforts. The lower cost and increasing availability of newer molecular tools have led to wide-ranging basic studies on turfgrass pathosystems. The widespread use of PCR and DNA sequencing facilities has made more precise, genetics-based pathogen identifications much more commonplace. Also, increasingly accessible next generation sequencing technologies have now made the sequencing of a pathogen’s entire genome highly feasible. Other advances in the genetic manipulation of pathogens, including more robust transformation systems, have been adopted from other crops’ pathosystems and applied to study numerous turf pathogen interactions. This has permitted the development of transgenic pathogens that can be used to tag pathogens with visual markers such as fluorescent proteins that facilitate the observation and elucidation infection processes at the cellular level. Adoption of this latter tool relies on increasingly common epifluorescent and confocal microscopy facilities. Future applications of molecular tools to turfgrass pathosystems will be discussed.

Using social media in turfgrass disease management education

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Phytopathology 101:S211

Traditional forms of communication can take a relatively long time to produce but nowadays there is an explosion of digital media outlets (e.g., blogs, facebook, twitter, others) as a communication tool that allows for rapid dissemination of timely information and encourages reader feedback and comments. In 2009, a website about turfgrass diseases (www.turfdiseases.org) was developed using Blogger. To make the information available in a broad range of formats, the website content was integrated with other social media platforms including Facebook and Twitter. Information to the different sites is posted as follows: 1) original blog updates are posted to the blog at turfdiseases.org; 2) blog links, photos, and other turf disease-relevant content are posted to Facebook; and 3) all blog and facebook
to the low infectious dose of E. coli O157, identification of bacterial and plant shredding lettuce compared with 2-fold on intact leaves within 10 h only. Due by human pathogens. Populations sizes of E. coli O157 increased 11-fold on plant lesions thus creating new niches for opportunistic colonization of leaves predominantly causal agent of these outbreaks. Harvesting and processing cause linked outbreaks of foodborne illness and Minimally processed leafy vegetables are the biggest culprits in produce-perspective on the transmission of these organisms. The historical association with animals and animal products has led to both organisms being labeled as zoonosis – infections transmitted by animal vectors to humans. Subsequently, the presence of these pathogens on fruits and vegetables has often been viewed by the public health and food safety communities as the result of contamination due to contact with feces from domestic or wild animal source at some stage during cultivation or handling and resulted in statements like “We’re trying to find out in what point in the process there might have been feed lot contamination”. A growing body of evidence, related to the role of the non-host environment in the life cycle of each organism, is being assembled. This presentation will discuss what is currently known about the frequency, prevalence, and behavior of Salmonella and EHEC in the produce production environment and the challenges in altering the historic perspective on the transmission of these organisms.

Escherichia coli O157:H7 persistence on plants: Lessons from the study of phyllosphere microbiota

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Phytopathology 101:S212

The aerial portions of plants are colonized by bacteria in amounts up to 10^6 cells per gram plant tissue. Molecular genetic studies of the phyllosphere residents have shown that these bacteria adapt for growth and survival in this habitat. Leafy green produce has been increasingly associated with outbreaks of human illness due to contamination of plants with human pathogens in the field prior to harvest. Although enterohemorrhagic Escherichia coli O157:H7 (EcO157:H7) is generally not a good colonist of the phyllosphere, it is able to survive and grow on lettuce in the field. We are investigating the influence of the indigenous phyllosphere-associated microbiota on EcO157:H7 colonists in relation to other environmental factors which influence pathogen adaptation for survival on Romaine lettuce. In field trials examining survival of an attenuated, non-pathogenic EcO157:H7 on Romaine lettuce in the Salinas Valley, California, total culturable population sizes of bacteria were typically inversely correlated with EcO157:H7 persistence on the plants, and certain phyllosphere isolates were shown to inhibit the growth of this pathogen in vitro. Phyllosphere bacteria were identified during different growing seasons, stages of plant maturity, and irrigation regimes by 16S rRNA gene sequencing using the Roche-454 pyrosequencing platform. These results show a large diversity of organisms with seasonal and irrigation dependent differences and correlations among plants inoculated with EcO157:H7.

Transcriptomic insights into the interaction of E. coli O157:H7 with lettuce

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Phytopathology 101:S212

Minimally processed leafy vegetables are the biggest culprits in produce-linked outbreaks of foodborne illness and E. coli O157:H7 (EcO157) is the predominant causal agent of these outbreaks. Harvesting and processing cause plant lesions and new niches for opportunistic colonization of leaves by human pathogens. Populations sizes of EcO157 increased 11-fold on shredded lettuce compared with 2-fold on intact leaves within 10 h only. Due to the low infectious dose of EcO157, identification of bacterial and plant attributes that enable or inhibit its growth on fresh-cut leaves is essential for the development of effective control strategies. Microarray transcriptomics and QRT-PCR revealed that EcO157 cells exposed to injured lettuce leaf tissue upregulate a wide range of virulence factors as well as genes involved in oxidative and osmotic stress, and antimicrobial resistance. Although multiple transport systems for carbohydrates that are prevalent in plants were activated in EcO157 in lettuce lysates, growth rates of the pathogen in lysates of different lettuce varieties were independent of carbohydrate concentration but correlated negatively with ROS concentration. In shredded lettuce leaves, expression of plant basal defense genes was affected by the presence of EcO157 compared with uninoculated leaves, indicating that a complex network of plant response to injury and colonization by the pathogen may modulate the outcome of a contamination event.

Hunting the plant essential Salmonella enterica genes

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Phytopathology 101:S212

Salmonellosis the most common bacterial food-borne illness contracted by Americans, and contaminated produce is a growing source of infection. Salmonella enterica is an animal pathogen that has recently been shown to colonize plants, meaning S. enterica can adhere, survive, and replicate on the plant. These findings have increased the urgency to understand the mechanisms used by enteric pathogens to colonize the plant niche. As fewer agricultural sites are isolated from the impact of urban development (e.g., waste, contaminated water supplies, etc.) produce cannot be ignored as a source of infectious diseases. Further, Salmonella persistence on produce can be considered a model system to better understand adaptation strategies a pathogen can use to survive in multiple niches, and thus maximize the probability of encountering a susceptible host. Through an omics approach, we have found no significant overlap between genes required for plant colonization and those required for infection of animals. A detailed understanding of the mechanism of S. enterica persistence will contribute to efforts to design intervention strategies to remove this and other pathogens prior to human consumption.

Insights from the comparative genomic analysis of pathogenic plant endophytic and clinical Klebsiella pneumoniae isolates

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Phytopathology 101:S212

We reported the first genome and comparative genomic analysis of a nitrogen-fixing plant endophytic Klebsiella pneumoniae species, strain 342 (Kp342). Kp342 serves as a model for studies of endophytic, plant-bacterial associations, due to its efficient colonization of plant tissues (including maize and wheat, two of the most important crops in the world) while maintaining a mutualistic relationship, which encompasses supplying organic nitrogen to the host plant. Although Kp342 is a member of the enteric bacteria and genome analyses identified a complement of genes involved in animal pathogenesis, when tested in mouse models, this species was less virulent in comparison to a closely related clinical strain. A comparative genomic analysis examined Kp342 for the presence of previously identified genes from other bacteria known to be involved in colonization of, or growth in, plants. From this set, approximately one-third of genes were identified in Kp342, suggesting that additional factors most likely contribute to its endophytic lifestyle and genome analyses were used to provide new insights into this question. I will present fresh data on comparisons with newly available genomes using a new pan genome analysis pipeline.

Does pectolytic activity of phytopathogens enhance Salmonella proliferation in tomato fruits?

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Phytopathology 101:S212

The presence of phytopathogens increases growth of Salmonella in plants. Uncovering the mechanisms by which Salmonella benefits from this
association would be another step towards pathogen-free fruits and vegetables. The published genomes of Salmonella do not contain recognizable pectinases. We hypothesize that pectinolytic activities exerted by phytopathogens provide additional nutrition to Salmonella. Extracellular degradation of pectin results in oligomers that Salmonella could potentially uptake as a carbon source. To test this hypothesis, Salmonella was cultured in M9 minimal medium with polygalacturonic acid (major component of pectin) as the carbon source in the presence of either pectinolytic enzymes or the soft rot phytopathogen Pectobacterium carotovorum. Salmonella CFU counts were 100 fold higher in M9 medium with polygalacturonic acid when grown in the presence of Pectobacterium. The presence of polygalacturonase in M9 with polygalacturonic acid promoted Salmonella growth. A similar benefit was not found when peptate lyases were added to the polygalacturonic acid containing medium.

Phytopathological Phreakonomics

The Freakonomics of plant protection

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Phytopathology 101:S213

Freakonomics by Levitt and Dubner presents several examples of the “Law of Unintended Consequences” in everyday life. In this session, we focus on freakonomics in plant protection—cases when the best intentions have led to unintended consequences. The first presentation describes the academic history of the “Law of Unintended Consequences” in neoclassical economics, beginning with Jevon’s Paradox, and some contemporary examples in crop protection. The presentation then summarizes the general economic and agronomic principles underlying these outcomes in crop production and suggestions for improving the science of plant protection and crop production.

How IPM contributed to the current fungicide resistance crisis in apple management

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Phytopathology 101:S213

Persistent use of single-site inhibitor fungicides to control of apple scab has led to multiple fungicide resistances in Venturia inaequalis. Fungicide resistance has developed with varying frequencies in orchards in the Midwest. In a recent survey, 38% of isolates were either resistant or shifted toward resistance to two fungicide classes, and 12% were resistant or shifted to all four fungicide classes examined (benzimidazoles, sterol inhibitors, strobilurins, and guanidines). The presence of resistance to all four of the single-site inhibitor classes commonly used for apple scab management has forced growers to revert to higher doses and more applications of old (1950s) protectant fungicides. Growers lose management flexibility and risk increased crop loss as they revert to old fungicides. Nearly 100 years ago, a physician named Paul Ehrlich (1913) recognized that it is best to “hit hard and hit early” to prevent microbes from developing resistance to treatment. This tenet conflicts with the fungicide reduction strategies that have been widely promoted over the past 40 years as integral to Integrated Pest Management (IPM). The IPM focus on pesticide reduction generated short-term “successes” without appropriate analyses of the long-term costs for managing plant pathogens. We provide multiple lines of evidence that the approaches used to implement IPM have contributed to fungicide resistance problems and may still be driving that process in apple production.

Don’t bother me with the facts: Strobilurins and plant health

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Phytopathology 101:S213

Strobilurin fungicides belong to the QoI fungicides. QoI stands for quinone oxidoreductase inhibitors. Recently, the use of fungicides that contain strobilurins has dramatically increased in corn and soybean production. This chemical group has also been touted as having plant health benefits. Some of the plant health benefits or claims include improved plant tolerance when stressed by abiotic factors. Also, in corn, plant health benefits have been touted for improved stalk strength or tolerance to damage from hail. While many physiological changes have been documented in controlled trials like those of greenhouse or growth chamber experiments, there exists little empirical evidence of such effects in the field. In this talk, emphasis will be on describing potential physiological changes due to the application of strobilurin fungicides followed by a closer examination of field trial results for corn and soybean. In particular, we will examine data from both published and unpublished work that examines the effect of hail damage or response to fungicide application in low disease environments. Overall, results from several years of field crop trials have indicated that the best response for use of fungicides is when the potential for disease is greatest.

Regulating the ubiquitous

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Phytopathology 101:S213

Detection of initial introductions of any exotic pathogen is challenging because by definition, exotic introductions occur in very low incidence. Initial surveys may underestimate incidence or may determine that pathogens have become dispersed across large regional areas and have become widespread, i.e., ubiquitous. Because exotic pathogens initially may not be found in all agricultural regions where a commodity is grown, they fall under strict regulatory authority and prompt swift action. Regulatory agencies require methodologies to quickly find and accurately delimit exotic pathogens. These methods often involve considerable physical and manpower resources. As pathogen incidence and the proportion of total infected commodity area increase, regulatory agencies must quickly adapt their mitigation strategies, even though changing regulatory policy can be hampered by political momentum and challenged by litigation. Regulatory agencies are becoming ever more reliant upon rapid regional survey methodologies and predictive models to estimate disease increase and spread that are linked to economic models to estimate changing fiscal and manpower resources. The need for such tools increases as the number of exotic pathogens assailing U.S. agriculture escalates. These tools provide the means to justify modifications to regulatory policy. Examples are provided from regional epidemics of the arboresc algae pathogens citrus canker, Huanglongbing, and plum pox.

Panacea or villain: Biocontrol is neither

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Phytopathology 101:S213

Although biological control has been effective in controlling a range of plant diseases, inconsistency in performance has plagued efforts to harness biocontrol for broad-scale commercial use. With this in mind, several quantitative reviews (meta-analyses) of published and unpublished studies have attempted to reach a consensus on the overall efficacy level of biological control across a spectrum of diseases, and to identify study factors (moderator variables) that influence the success or failure of biocontrol. Taken together, these analyses showed that biological control is moderately but significantly effective overall, and that study-level moderator variables such as disease pressure, host plant type/cultivar, or nitrogen level/form can explain some of the variability observed across individual studies. The meta-analyses also revealed significant interactions between pathogen and biocontrol agent type on biocontrol efficacy. For example, r-selected biocontrol agents were more effective than those that were not r-selected; products based on Bacillus subtilis performed better against fire blight in the eastern U.S. than those based on Pantoea agglomerans; and mycorrhizae were more effective biocontrol agents than rhizosphere bacteria across a range of pathogen-host combinations. Some of these findings are at odds with conventional wisdom, warranting further research into the mechanisms underlying the observed moderator variable effects.

Against the current: Pests, pathogens, and produce on the St. Lawrence Seaway

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Phytopathology 101:S213

On May 3, 1959, residents of Duluth MN and representatives of the major exporting industries in the Upper Midwest gathered at the city’s iconic aerial lift bridge canal to welcome the first ocean-going ship to traverse the entire length of the newly completed St. Lawrence Seaway. At the time, the $639 million Seaway promised to breathe new economic life into the cities and towns that lined its 2500 mile drift from Montreal to Duluth, and for a while it did. Twenty years later, however, it had become painfully obvious to some that the Seaway was not only a marginal economic venture but an ecological disaster. Both concerns continue to this day. Over 150 invasive aquatic pests
and pathogens originating beyond North American shores have found travel on foreign vessels convenient for mounting an invasion into previously (for them) uncharted territory. And plant health care professionals educated in the mid-20th Century who must have scoffed at their predecessors’ careless introductions of Dutch elm disease, white pine blister rust, and chestnut blight are left now to cope with the Seaway’s latest round of terrestrial foreign invaders. Asian long-horned beetles and emerald ash borers get the headlines, but one has to wonder just how much more trouble is yet to surface ... and at what cost. Scrupulous surveillance and prompt eradication seem to provide the only viable options for minimizing future damage. Or, as some have suggested, we could just give up.

Schroth Faces of the Future in Nematology
Chemical ecology and isolation of biologically active compounds from parasitic nematodes

Root knot nematodes (Meloidogyne spp) are possibly the economically most important and best-studied species of plant parasitic nematodes. However, for Meloidogyne spp. and the intensely studied nematode, Caenorhabditis elegans, very little is known about signaling within and in-between species. It has been reported that Meloidogyne species prefer uninfected over infected roots when given a choice. However, the nature of this signal is unknown. When studying C. elegans behavior, we discovered the composition and release of nematode produced small signaling compounds to be tightly correlated with environmental conditions such as crowding and food availability. These signals lead to transition into and out of the dauer stage, which is equivalent to infective juvenile stage of plant parasitic nematodes. We are taking two approaches to identify chemical cues mediating host attraction and avoidance: First, by collecting and bioassaying of volatile cues from Meloidogyne incognita infected and uninfected tomato roots, second, by collecting and bioassaying water soluble cues from exudates and root extracts of plants infected with nematodes and from healthy roots. The metabolites from infected roots and root exudates are fractionated by various chromatographic methods and tested for biological activity. Results from the second approach will be presented.

Teaching and learning plant-parasitic nematode identification
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Training nematode identifiers is just as essential to ensure a healthy future for the field of nematology as is the education of nematode systematists. Training nematode identifiers is very different from forming nematode taxonomists. Identifiers rely on practical criteria and are expected to make routine identifications at high speed and with light microscopy. I will share my experience as a trainer of identifiers in Clemson University, where I host a one-week course on Plant-parasitic Nematode Identification. Course participants typically include research and extension scientists, professional consultants, regulatory personnel, diagnos-ticians, and graduate students. The course is structured as short lectures on the biology and ecology of the nematode genera, immediately followed by direct observation of specimens. The emphasis of all the teaching materials is on identification by morphology. The “recognition” approach is favored, where all relevant features of a specimen are used simultaneously to identify the genus, as opposed to the sequential approach that taxonomic keys dictate. Participants prepare and observe their own mounts of fresh specimens of the 25 most common plant-parasitic nematode genera in agricultural soils. A brief history of the course is presented along with a discussion of the evolution of the teaching techniques.

Dissecting the interactions between Meloidogyne chitwoodi and potato – an integrated approach
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The Columbia root-knot nematode (Meloidogyne chitwoodi) is a major pest in potato in the Pacific Northwest and has quarantine status in many countries. Our overall objective is to understand the molecular interactions between plant-parasitic nematodes and their host plants. Using the M. chitwoodi-potato pathosystem as an example, we will dissect plant-nematode interactions and develop novel control strategies utilizing an integrated approach. Effector genes play a key role in molecular plant-nematode interactions and will not only advance our understanding of fundamental processes in nematode parasitism, but might also be exploited as control targets. In addition, our lab employs comparative genomics to gain a better understanding of how plant parasitism evolved in Meloidogyne. At present, four different M. chitwoodi populations are known that differ in their host ranges and their ability to overcome resistance genes from a wild Solanum species. We are studying the genetic and morphological variability between these pathotypes and their interactions with host plants and will discuss how pathotype diversity impacts our ability to develop molecular control strategies that are effective against all M. chitwoodi populations. We hope to provide evidence that the future of nematology lies in addressing nematode disease problems in an integrated manner and benefits from combining molecular and classic techniques and asking questions in an ecological context.

Technology Outlook: Detection Innovations and Successes
Deployment of DNA arrays in plant pathogen detection
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DNA arrays have been around for over a decade now and are being used in some commercialized diagnostic applications. For functional genomics and microbial ecology studies, the use of DNA arrays has been generally superseded by next generation sequencing, mainly because arrays provide data on known pathogens and can be prone to misleading results if unknown organisms closely related to the targets are in the sample. However, there are niches where lower density DNA arrays are still a more economical and practical tool to generate data on the presence/absence of a broad range of specific plant pathogens. Arrays do provide the potential of running large number of samples in a cost effective manner. There are high throughput array based platforms developed for human pathogens that could be adapted for plant pathogens. The first lab on a chip for plant pathogens was designed for Phytophthora. It uses an array system whereby a positive hybridization event closes with silver a micorocircuit gap located over a micro spot of specific oligonucleotides. The best way to develop reliable arrays and diagnostics in general is to include a large proportion of species from the target genera in the design and validation stages, even if the assays are being developed only for a few selected species. Therefore, the availability of comprehensive collections of specimens or strains and high quality sequences associated with them remain at the core of any diagnostic assay development.

/PhyClassifier: An interactive online tool for phytoplasma identification and classification
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Phytoplasmas are phloem-inhabiting, cell wall-less bacteria responsible for numerous diseases in agriculturally and ecologically important plant species. In recent years, new phytoplasmas have been discovered at an increasingly rapid pace in emerging diseases worldwide. Correct identification of diverse phytoplasma strains is the key to accurate disease diagnosis and epidemic management. Since phytoplasmas cannot be cultured in cell-free media, identification and classification of phytoplasmas have been primarily based on molecular analysis of evolutionarily conserved gene sequences. Recently we constructed an interactive online tool, /PhyClassifier, to expand the efficacy and capacity of the current 16S rRNA gene RFLP-based phytoplasma classification system. /PhyClassifier performs sequence similarity analysis, simulates laboratory restriction enzyme digestions and subsequent gel
electrophoresis, and generates virtual gel images. Based on RFLP pattern similarity coefficients and sequence identity scores, iPhyClassifier makes instant suggestion on group/subgroup classification status and ‘Candidatus Phytoplasma’ species assignment for the phytoplasma under investigation. A newer version of the tool, iPhyClassifier 2.0, is currently being developed. The new version will include additional functions such as multi-locus marker analysis for finer differentiation of closely-related lineages, and composite RFLP pattern display for identification of strains with sequence-heterogeneous RNA operons.

**Using surface plasmon resonance (SPR) technology to detect quarantine plant pathogens**

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Rapid and accurate detection of quarantine pathogens is needed to safeguard our agriculture and natural resources. Surface plasmon resonance (SPR)-based molecular detection methods can fulfill this need. SPR technology is based on the use of a biosensor that utilizes a capturing molecule (the ligand) as a reactive surface in close proximity to a transducer (e.g., a gold film) which converts the binding of an analyte (the pathogen) to the ligand into a measurable signal. SPR is currently used to detect food-borne bacteria and toxins. A handheld SPR device is being explored to be used for detecting quarantine plant pathogens. *Ralstonia solanacearum* (Race 3 biovar 2), a quarantine pathogen in the U.S. that is of high consequence and importance has been chosen as the first study subject. The use of *R. solanacearum*-specific antibody and other molecular probes targeting to different races and biovars of *R. solanacearum* will be discussed for the detection of this important quarantine plant pathogen.

**The quest for unknown viruses in plants by siRNA deep sequencing**

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Novel and emerging plant viruses, driven by pathogen evolution, global trade, crop intensification and potentially climate change, pose a key threat to agriculture worldwide. Even apparently symptomless virus infections can cause considerable yield losses, which can be further exacerbated by synergistic interactions with other viruses. RNA silencing constitutes a fundamental antiviral defense mechanism in plants in which host enzymes cut viral RNA into pieces of 20-24 nt. When isolated, sequenced en mass and properly aligned these virus-derived small RNA (sRNA) sequences can reconstitute genomic sequence information of the viruses being targeted in the plant. This approach is independent of the ability to culture or purify the virus and does not require any specific amplification or enrichment of viral nucleic acids as it automatically enriches for small RNAs of viral origin by tapping into a natural antiviral defense mechanism. To date the method has been used to identify numerous new viruses including single and double stranded RNA, DNA and reverse transcribing viruses from hosts as divergent as plants and invertebrate animals. In several cases unexpected apparently symptomless viruses were also identified, providing an important reminder that there may be many more viruses infecting our crop plants than we have previously been aware of, and their potential impacts on agricultural productivity remain to be understood.

**The use of isothermal DNA amplification (NEAR) in plant disease diagnostics**

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The nicking enzyme amplification reaction (NEAR) is an example of an isothermal amplification technology that offers a PCR inhibitor resistant solution in a rapid, sensitive and specific format to screen plant samples for the presence of pathogen causing disease. NEAR isothermal amplification technology can amplify and detect a specific plant pathogen in less than 14 minutes to as fast as 2 minutes in a real time format. We will discuss matrix effects on the efficiency of real time NEAR assays for *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the causal agent of bacterial canker in tomatoes, and *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of potato ring rot. Our data shows specific detection of Cmm and Cms directly from infected tissue using a simple maceration of the tissue and confirms the desired specificity. The results indicate the possibility of detecting plant pathogens and disease in very early stages of development prior to the onset of symptoms. In addition, we will present data for the detection of both Cmm and Cms from samples using a real time NEAR fluorescent format as well as on a DNA lateral flow strip housed in self-contained handheld device. NEAR is a robust assay, with little inhibition, allowing for amplification directly from crude extracts without further purification. The exquisite assay sensitivity, speed and portability make it an ideal choice for point-of-testing (POT) and field analysis.

**Tropical Forest Pathology**

Diseases of tropical *Eucalyptus* spp.: Growing threats to a critically valuable global forestry resource

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*Eucalyptus* spp. have been propagated outside their native range for more than a century. However, it is only during the course of the last three decades that these trees have been widely planted in the tropics. This has led to substantial experimentation with tropical species that were not propagated in plantations during the earlier part of the 20th Century. A major consideration in developing suitable species has been to avoid disease problems that have increasingly grown in importance. These diseases have emerged through the accidental introduction of pathogens to new areas. But interestingly, some of the most important diseases are caused by remotely host specific pathogens undergoing host shifts from native Myrtaceae in the tropics. These unexpected new host encounter diseases cause by pathogens such as *Puccinia psidii* and various species in the *Cryphonectriaceae* have now also begun to move to new environments. The consequences are a eucalypt resource that is increasingly threatened by disease and in some areas, long term sustainability has been questioned. Against this worryin g backdrop, application of new technologies including hybridization, genetic modification, knowledge emerging from the recently sequenced first *Eucalyptus* genome and tools to better understand pathogen biology and genetics provide opportunities to reduce the impact of diseases in the future.

**Current knowledge of Eucalyptus rust in Brazil**

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*Puccinia psidii* is expanding its geographic and host range, exemplified by recent reports in Hawaii, Japan, and probably in Australia. On eucalypts, infection occurs on leaves and shoots causing necrosis, loss of apical dominance, hypertrophy, and deformation of the infected organs. Under controlled, dark, conditions, infection takes place at 10 to 25°C (optimum 23°C) with 648 h of leaf wetness (optimum 24 h). Infection does not occur below 10°C or above 30°C. Yellow urendial and, more rarely, brown telial pustules are formed on infected organs. In the field, periods longer than 6 h with RH = 90% at 1825°C favor disease development. A recent study indicated that rust intensity decreases as plant height increases due to a reduced duration of leaf wetness and a reduced concentration of airborne urendinospores at greater heights. High inter- and intra-specific genetic variability within *Eucalyptus* spp. allows selection and planting of rust-resistant genotypes. In a specific *E. grandis* family, rust resistance is controlled by a single major locus, *Ppr-1*. A further study positioned the *Ppr-1* gene on the reference genetic map for *Eucalyptus*, and the results were consistent with the hypothesis that *Ppr-1* controls a large proportion of resistance. In families of other species and distinct genetic backgrounds, the genetic basis of resistance is more complex. Furthermore, the genetic variability among the pathogen populations is also a major concern in resistance screening. To date, extensive sampling has revealed considerable genotypic diversity among *P. psidii* isolates, as well as differences in pathogenicity on diverse host species.

**Invasion of Puccinia psidii into Hawaii, hosts infected, molecular characterization, and pathogenicity tests**

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*Ohi’a rust* caused by *Puccinia psidii* was first discovered on infected *Ohi’a* on the island of Oahu in 2005. Since then it has spread to all of the major Hawaiian Islands and has been found infecting 21 Myrtaceae hosts. New
sustainable hosts continue to be found, and the spread of the disease into new areas is eminent. Molecular analysis of the rust was expanded with the addition of newly developed primers. The genotype of the rust in Hawaii remains similar to a strain from Florida and from the intercept of myrtle from California. To determine host range and of the rust, pathogenicity tests were initiated in 2011 on: rose apple (Syzygium jambos), wax apple (Syzygium samarangense), ohia (Metrosiderous polymorpha), and eucalyptus (Eucalyptus torreliana). Two weeks after inoculation all hosts were infected. Additional Myrtaeae hosts, allspice (Pimenta dioica), mountain apple (Syzygium malaccense), guava (Psidium guajava), and Surinam cherry (Eugenia uniflora) will also be tested. Results of the host range test show that the Hawaii isolate has a wide host range, highlighting the risk to the native Hawaiian forest.

**Disease resistance screening for Koa wilt disease**

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The wilt disease of Acacia koa (koa) due to infection by Fusarium oxysporum f. sp. koae causes high rates of mortality in field plantings and threatens native koa forests in Hawaii. Identifying and developing wilt resistant koa populations may be the key to successful koa reforestation and restoration. The objectives of this research were to screen koa seedlings for resistance and susceptibility to Fusarium wilt and to determine if resistance is correlated with levels of expression of different classes of chitinases. We have isolated complete cDNAs encoding four different classes of chitinases from koa. In greenhouse experiments, seedlings grown from seeds of selected koa trees were screened for resistance and susceptibility using a mixture of virulent F. oxysporum isolates as the inoculant. Koa families with relatively high frequencies of resistant progenies were grouped as ‘resistant’ while families with high frequencies of seedling mortality were grouped as ‘susceptible’. We are now testing the half-sib progenies from resistant and susceptible families for expression levels of the chitinase genes using quantitative PCR. Expression levels of the chitinase genes may provide an additional measure of wilt resistance of the koa seedlings that survive infection by virulent isolates of F. oxysporum.

**Decline of Casuarina equisetifolia (ironwood) trees on Guam: Ganoderma and Phellinus**


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Ironwood trees (Casuarina equisetifolia), on the island of Guam have been in a state of decline for the past ten years. To determine the status of the decline problem and to seek possible causes, a survey of 1427 trees was conducted. A highly significant (p = 0.0001) linear function (r² = 0.997) between the presence of conks and decline severity emerged. Sixty-five percent of the trees at the most severe level of decline (nearly dead) had conks. Species from five basidiomycete genera of the class Agaricomycetes, belonging to the orders Polyporales (Ganoderma, Favolus, Pycnoporus), Hymenochaetales (Phellinus) and Thelephorales (Sarcodon) were identified based on macro- and micromorphology and DNA sequencing. The most common species observed was in the genus Ganoderma. Diagnostics was based on the prolific production of double walled basidiospores from sporocarps (a characteristic feature of members of the Ganodermataceae). Nuclear ribosomal (ITS) DNA sequences confirmed Guam’s species as a member of the G. australis species complex. The second most frequently collected conk belonged to the genus Phellinus. These two known genera of Casuarina wood rotting fungi are most likely playing a prominent role in the decline of Guam’s ironwood trees. Due to the high association between levels of management and decline, it is believed that tree wounds from lawn equipment serve as a point of entry for the two fungi.

**Emerging Pests/Invasive Species**

**Ag and Food Biosecurity: A Decade of Progress and Reality**

Crop biosecurity: An international perspective

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Biosecurity is emerging as one of the most important issues facing the international community. Although traditionally associated with risks for humans and animals from infectious diseases and biologically weapon, it now includes risks to agricultural crops, natural ecosystems and food. Agriculture and associated upstream and downstream related sectors are essential to the social, economic and political stability of all nations. Agricultural systems, including the food supply chain, are global in nature and pose a relatively soft target for those intent on harm, offering various points at which commodities could be deliberately contaminated. Addressing biosecurity issues related to crops and food has generated new fields of research and emphasized the need for continued investment in traditional fields of plant biology. In a broad sense, crop biosecurity is aimed at protecting agricultural lands and forests as well as the food supply from the natural or intentional introduction, establishment and spread of plant pests, pathogens and noxious weeds.

Securing the agro-food system requires the ability to identify high priority threats and then to prevent, detect, respond to and recover from actual events. Due to the global nature of agriculture, an international approach is needed. Some of International projects on crop biosecurity will be described. In particular, a Network of Excellence on Crop and Food Biosecurity, recently funded by the European Commission, is described.

**Global insect threats and issues for agricultural biosecurity**

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The issue of global insect threats is one of the most important current global topics in agricultural biosecurity. Interest does not just stem from reasons of avoiding the introduction of devastating insects from one geographical region to another, but also the damage, social issues, animal and crop losses, economic shortfalls and losses in biodiversity as a direct consequence of insect invasion. Recent increases in global food prices in many countries and shortages of food in others, calls for a crucial need to understand the importance of insect threats, how they are related to international trade, and
the appropriate measures needed to overcome the problem. It is critical to understand, not only on agricultural biosecurity related issues, but also the approaches on agricultural pest risk assessment, how they are developed and applied globally in different regions and continents. The procedures and the roles of various national, regional, and international organizations such as the World Trade Organization and regional Plant Protection Organizations will be discussed as well as some policy issues when applying national and international rules in export and import of agricultural products and goods. In that context, it is equally important to know the international rules and regulations and also issues on trade, trade barriers and potential trade protectionism.

Food defense: Farm to fork

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History has demonstrated that the food chain is a relatively easy target for a bioterrorist. The modern day food chain is highly complex with raw and finished products being sourced from multiple sources. The open nature of food chains makes defence against natural or deliberate contamination impossible. Yet, risk analysis tools, such as CAVAR-Shock, that are available to rank the various scenarios in terms of likelihood, impact, recoverability and psychological affects on the population. By identifying the most vulnerable points it is possible to identify foods and points in the chain to focus resources. Biosecurity programs and diagnostic devices also play a role in guarding the food chain or making targets less attractive to attack. More recent innovations are the establishment of intelligence databases that can be accessed by agencies across the globe to identify potential threats. The innovations to protect the food chain from attack by bioterrorists can also enhance safety against the natural introduction of biohazards and these will also be covered in the presentation.

Microbial forensics: Investigative plant pathology

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Microbial forensics is currently seen as a new field that overlap functions with biosecurity, biosafety, microbiology, and plant pathology. Microbial forensics is a scientific discipline that analyzes microbial activity as evidence for attribution purposes and/or tracing back to a point of origin. Microbial forensics performs into a rigid legal frame and demands a rigorous (accredited) and unbiased performance. Typical investigations may include the application of discriminatory methods to determine the cause of crop injury, herbicide damage, phytotoxicity, identification of microbes and insect during new incursions, track back of exotic microbes, investigation of introduction pathways and microbes associated to biocrimes during criminal investigations, etc. The methods and techniques used are similar to those applied in plant pathology, but microbial forensics demands further exhaustive testing and supportive unbiased accreditation systems. In microbial forensics maximum sensitivity is necessary as well as specificity. In order to reach high specificity and discrimination capability, all new assays need to be challenged through broad inclusivity and exclusivity panels seeking unequivocal identification of the target. The new techniques to be proposed for investigative purposes have to be robust to pass the strict scrutiny at the court of law.

The dual use dilemma

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In the international market place seed is routinely moved among countries for the purposes of research and breeding, production of stock seed and seed increase (counter season production), and commercialization. Before seed is allowed entry into a given country, it must first meet the phytosanitary import requirements of that country. In the seed industry, re-export of seed from one country to another is a major issue; seed may pass through several countries before reaching its final destination. Phytosanitary measures for seed pests currently are not harmonized internationally, which results in significant trade disruptions. Of major concern are seed borne and seed transmitted pathogens. This presentation will discuss the international nature of the seed industry, current phytosanitary concerns of seeds, issues associated with seed as a potential pathway for the introduction of unwanted pathogens and other pests into new environments, and progress toward resolving phytosanitary trade barriers through the development of trade agreements and regional and international standards.

Introductions of exotic insects and their associated pathogens in solid wood packing material

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Recent studies in North America and Europe have highlighted the mounting problem of importation of exotic forest pests in solid wood packing material (SWPM). With an increase in international shipping, there has been an increase in the detection and establishment of exotic phloem and wood boring insects in crating material, pallets, and dunnage. Establishment rates of forest defoliating and plant sucking insects have apparently leveled off in the U.S.A. with increased quarantine regulations and inspections of live plants. However, establishments of phloem-feeding bark beetles (Curculionidae: Scolytinae) and wood boring insects (especially scolytid ambrosia beetles) have increased in recent decades in the U.S.A. and Europe. China and other major exporting countries in Asia have become a major source for wood boring beetles, especially ambrosia beetles and cerambycids. The Asian longhorn beetle (a cerambycid) and the emerald ash borer (a hyporist) are noteworthy recent
arrivals from Asia. Relative to the ‘live plant’ pathway, few exotic forest pathogens have been associated with SWPM, but they have had dramatic impact. The related Dutch elm disease and laurel wilt pathogens have exotic bark beetles and ambrosia beetles, respectively, as vectors. Introduction of *Ceratocystis platani* to Europe from the U.S.A. was likely facilitated by the boring frass of ambrosia beetles in SWPM. Insects and their fungi protected in wood used in commercial trade continue to be serious biosecurity threats.

**Flaws in international protocols for preventing entry and spread of plant pathogens via “plants for planting”**

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Numerous ‘exotic’ tree and plant pathogens have been arriving in Europe, North and South America, and elsewhere, mainly as a consequence of increasing globalisation of trade in rooted plants and a serious flaw in international plant health (SPS) protocol. Unnamed organisms cannot be legislated against, yet up to 90% fungal pathogens in underexplored ecosystems may be unknown to science. In Europe the problem is compounded by a range of weaknesses in execution of plant health measures such as visual inspection of only ~2% of imports, infested but asymptomatic stock being given ‘clean health’ certificates, and failure of states to report new pests. Essentially, the European plant health system is overwhelmed and most UK trees, for example, must now be considered at high risk. *Phytophthora ramorum*, currently epidemic on oaks in the U.S. and on larch in the UK, is a symptom of these problems. The status quo is not an option: modernisation of global plant health protocol in line with the risk from unknown organisms is needed. Intensifying diagnosis at ports is unlikely to stem the tide. Options include shifting onus onto exporting countries, reducing high volume / high risk ornamental imports, importing only seed, small numbers of plants or tissue cultures under quarantine for local propagation under licence; certifying nursery stock as disease free at point of sale; and educating media, trade and public on the environmental costs and consequences of these damaging introductions.

**Progress and pitfalls in developing policies for reducing risks of introductions of exotic forest insects and pathogens**

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Nearly 500 species of arthropods and pathogens have become established in North American forests. While the overall rate of introductions of forest pests has remained steady, detections of “high-impact” and wood-boring insects associated with wood packaging have increased significantly. The most promising remedy is to manage introductory pathways so as to minimize pest presence in the imported goods or packaging. The pace of applying this concept varies greatly among pathways. The first international application of this approach dealt with wood packaging. Unfortunately, it has reduced the insect approach rate far less than expected. Phytosanitary officials now need to identify and correct the problems. North America has made rapid progress on a second pathway – preventing Asian gypsy moths from laying eggs on ships. Trade in living plants has a long history of damaging introductions. Phytosanitary officials at the national, North American, and international levels have repeatedly endorsed replacing visual inspection at the border by “systems approaches”. Nevertheless, most plants continue to enter the U.S. subject only to visual inspection. Inside the country, USDAAPHIS is struggling with movement of pests in firewood and on nursery plants (e.g., *Phytophthora ramorum*). Stakeholders have formed the Continental Dialogue on Non-Native Forest Insects and Diseases to voice their concerns.

**Digital Identification Tools: Their role in Biosecurity and Pest Management**

Designing, developing, and delivering digital identification tools for plant protection and quarantine

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The USDA-CPHST Identification Technology Program (ITP) supports Plant Protection & Quarantine (PPQ) in its efforts to prevent the entry into and establishment in the U.S. of invasive pests, diseases, and weeds by delivering digital identification tools and resources. Our challenge is to address new identification challenges and embrace advanced technologies in order to maximize identification capabilities within PPQ. Our clients are diverse—in their level of experience, educational background, as well as their detection and identification responsibilities. We thus strive to serve both a broad audience and to address specific current and future needs of our clientele. ITP continues to learn that our clients require rather unique and specialized tools to support their particular identification process. Therefore, the design, layout, and functionality of digital tools are based on the background knowledge obtained about the clients identification responsibilities, educational background, level of expertise, and level of reporting required by the client (actionable, non-actionable, detection, verification, etc.). The various types of digital tools and resources recently designed and delivered by ITP for meeting specialized client needs and those developed for broader groups of clientele will be summarized with respect to content, functionality, value, and usefulness for the end user. Guidelines for addressing the design and development for a requested identification tool will also be discussed.

**The Pestnet diagnosis service in the South Pacific and Southeast Asia**

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Phytopathology 101:S218

Pestnet is a free advisory service that gives fast expert answers on all crop protection matters via email (pestnet@yahoogroups.com). This includes advice on crop diseases and quarantine matters, plus regular updates on research. Pestnet addresses the constraints that are associated with sustaining agriculture and forestry-based livelihoods, particularly when pests and diseases abound and there is no advice readily available. There are five volunteer moderators in Australia, Fiji, New Zealand, Thailand and Uganda. The service is available for farmers, extension workers and crop protection specialists worldwide. There are now 1000 members, representing some 80 countries. Over 10 years, nearly 8000 messages have been exchanged, and many of the discussions have been summarised and placed on the Pestnet website (http://www.pestnet.org/).

**The role of Q-Bank in supporting plant regulatory agencies**

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The rate of introduction and establishment of damaging plant pests and diseases has increased steadily over the last century as a result of expanding globalisation of trade in plant material, climate change, EU expansion, and by a recognised decline in the resources supporting plant health activities. Furthermore there is a constant decline in the number of taxonomic specialists in the different disciplines (mycology, bacteriology, etc.), capable of identifying plant pathogens (in particular new emerging diseases). Also other specialists in phytopathology and other fields which are vital for sustaining sound public policy on phytosanitary issues are threatened with extinction. These problems affect all members of the EU and other nations. In this context Q-bank has been developed (www.Q-bank.eu) and now consists of a dynamic open-access database of regulated plant pests and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections. DNA barcoding data of quarantine organisms generated in the EU project of QBOL (www.qbol.org) will be made available for Q-bank to support plant health diagnostics. Curators from many countries for the different groups have been appointed and links with other databases have been made; this in order to provide Q-bank an international role in supporting plant health agencies.

**PaDIL - A Virtual Diagnostic tool to assist in plant pest diagnostics**

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Fundamental to minimising the risks of pests crossing national or internal borders is to be able to rapidly diagnose them accurately and efficiently. Activities aimed at lowering pest risks need to know what species they are dealing with but the majority of pests encountered are not local; and, there is a worldwide decline in the availability of diagnosticians and taxonomists for plant pests. To address some of these issues, PaDIL (http://www.padil.gov.au), a Virtual Diagnostic tool was developed to harvest reference specimens from recognised Museums and Herbaria around the world with the view to building a Virtual Pests collection. PaDIL provides high quality, colour, diagnostic and symptom images (almost 40,000 images) and basic information for almost 2000 recognised plant pests species (i.e. taxonomy, distribution, hosts etc.). The interactive software allows users to Navigate and Explore the datasets and allows the user to create their own views/outputs to the results returned to their queries. PaDIL is freely accessible, requires no software downloads
and the images are free to use for non commercial use. The primary target audience of PaDIL is plant biosecurity diagnosticians with some level of experience; however, the image-based website can be easily used by specialists and non-technical users. PaDIL is an example of transitioning taxonomy from a Museum-based resource into an Information-based resource.

Leveraging digital resources and social networks for identification and extension education

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Phytopathology 101:S219
A wide variety of digital resources provide new avenues in plant biosecurity to help identify suspect organisms and retrieve relevant biological information. While many of these resources can be effective as stand-alone products, integrating these resources into a suite of options can better target the commodity interest, geography, and role in plant biosecurity of a specific audience. Further benefit can also be leveraged from these resources by making the individual images, fact sheets and videos available for the development of additional education materials. Maximizing the potential impact that a resource can provide to the plant biosecurity community requires collaboration between resource builders to provide effective resource linkages and efficient sharing of materials while providing appropriate credit to developers and providers. Development of these programs requires interaction with extension educators to understand their preferred types of educational resources, how resources can be improved to be more effective and different ways the raw resource components could be utilized for their audiences. Social networking services are a key component in efficiently delivering news of a tool’s existence, identifying potential partners for building new resources, determining appropriate outlets for distribution of the raw materials for use in related projects, and promoting collaboration to maximize the utility of all digital resources.

IPM and Biological Control of Insect Pests, Plant Pathogens, and Invasive Weeds in the Pacific Islands: Where Are We Heading?

Experiences with biocontrol of invasive pests and weeds in the Pacific Islands

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Biological control of invasive pests and weeds has been recognised as the best option for pest management in the Pacific Islands due to its sustainability, low environmental impact on fragile island environments, adaptability and low ongoing cost. It is an important component of most IPM programmes in the region. In the last five years, ongoing biological control programmes have included fruit flies (Bactrocera dorsalis, B. tryoni, B. kiriki) in French Polynesia; rhinoceros beetle (Oryctes rhinoceros) in Guam, Fiji, Samoa and Papua New Guinea (PNG); taro beetles (Papuana spp.) in Solomon Islands, Vanuatu, Fiji and PNG; water hyacinth (Eichhornia crassipes) in Fiji, Vanuatu, Solomon Islands, and PNG; and the coconut flat moth in Fiji, cycad Aspalachis scale in Palau and the glassy wing sharpshooter (Homalodisca vitripennis) in the Cook Islands. New programmes include the Erythrina gall wasp, (Quadrastichus erythrinae) in PNG and Fiji; bitter vine (Mikania micrantha) in Fiji, Guam and PNG and the cocoa pod borer (Conopomorpha cremenella) in PNG. Progress in these control programmes will be presented and the need for ongoing support and monitoring discussed.

Containing the rhinoceros beetle outbreak on Guam

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Phytopathology 101:S219
The coconut rhinoceros beetle (CRB), Oryctes rhinoceros, a major pest of coconut palms, was first found on the Micronesian island of Guam in September, 2007, at the center of the Tumon Bay hotel district. Adult beetles damage and sometimes kill palms when they bore into crowns to feed on sap. Tree mortality exceeded 50% several years after CRB invaded the Palau Islands, which are also in Micronesia and this level of damage may occur on Guam without intervention. Coconut is not a major crop on Guam, but palms are valuable ornamental plants for Guam’s hotel and tourism industry. Following a delimiting survey which indicated that the CRB population was localized along a five mile stretch of Guam’s northwestern coast, an eradication project was initiated. Tactics include local quarantine, mass trapping, sanitation, detector dogs, chemical control, and biological control. A short history of the project will be presented followed by a discussion of successes and failures for each tactic.

Behavior and management strategies for taro beetles in Pacific Islands

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Phytopathology 101:S219
Beetles of the genera Papuana and Eucopidocaulus (Coleoptera: Scarabaeidae) are the most serious pests of taro Colocasia esculenta in Papua New Guinea, Solomon Islands, Vanuatu, Fiji and Kiribati and are a quarantine threat to the neighbouring Pacific island countries where taro and related aroids are important staples. The adult beetles feed on edible underground corms and often concealed feeding inside the corms. The beetles often breed outside the gardens and invade the plants soon after planting, often killing immature plants. At least two pest species co-exist in the same location or islands in the native fauna except for Fiji and Kiribati where single introduced species exists. These coexisting species rarely feed on the same host plants and breed in the same habitats. This makes it difficult to identify and develop generic control measures. Adult male beetles often remain in the gardens and continue breeding on the developing plants while the females tend to feed and then go back to the breeding sites for oviposition. The adult beetles are sexually active in the first three months of their life span and therefore the females lay 70% of their eggs, while the remaining 30% is laid in the last 9 months. Adult beetles can live up to a year. In order to develop suitable control measures, the host preference and breeding habits of each species must be understood as measures developed for one species may not be effective against related species in areas where two species co-exist.

Pests of Oil palm in Papua New Guinea, with emphasis on West New Britain

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Phytopathology 101:S219
Oil Palm (Elaeis guineensis) is native to West Africa but was introduced into Papua New Guinea (PNG) for commercial planting in 1967. It is now PNG’s highest earning agricultural export, producing oils that are very widely used. Over 130,000ha are currently under oil palm with 12 processing mills supporting estates and 18,315 smallholder blocks (Dec 2009). PNG uniquely manufactures a traceable, sustainable palm oil adhering to the Principles and Criteria of The Round Table for Sustainable Palm Oil (www.rspo.org) and is also ISO14001 accredited. The commercial life span of an oil palm is about 20 years. As with other monoculture crops, and introductions to PNG, oil palm is attacked by many invertebrate, vertebrate and fungal pest taxa. Principle pests are Orthoptera (Tettigoniidae), Coleoptera (Scarabaeidae & Curculionidae), Lepidoptera (Psychidae), and Muridae (Rodentia) with few attacks from birds (Aves). One taxon of fungi (Ganoderma boninense) has a devastating effect on oil palm productivity. The management of pests is focussed on the principles of Integrated Pest Management, where chemical intervention is only used when populations reach threatening levels. At this stage supervised targeted trunk injection (TTI), using a systemic insecticide, is undertaken. Failure to treat pests in a timely manner can cause large financial losses due to reduced production which can take years to recuperate.

Effectiveness of fruit fly parasitoids introduced from Hawai’i in French Polynesia

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Phytopathology 101:S219
After five years of unsuccessful chemical campaign of oriental fruit fly control, the egg parasitoid Fopius arisanus and the third-instar larva parasitoid Diachasmimorpha longicaudata were introduced, in December 2002 and May 2007 respectively, from Hawai’i to suppress the three fruit flies species present in Tahiti (Bactrocera dorsalis, B. tryoni and B. kiriki ). Over a 2 year period since the date of their introduction, 300,000 Fopius and 21,000 Diachasmimorpha have been released at different sites on Tahiti. From 2003 to 2009, >500 kg of fruit were collected annually from around Tahiti, and stored in boxes to obtain pupae from which fruit flies or parasitoids will emerge. Over the monitoring period, the rate of Fopius emergence increased

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Wheat Blast—A Potential Threat to Global Wheat Production

Cellular and molecular defence responses of wheat to Magnaporthe species

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Phytopathology 101:S220

Blast disease has emerged as a serious field disease threatening wheat production. The causal agent of wheat blast, Magnaporthe oryzae, infects cultivated crops, while M. grisea infects wild grasses. Cellular defence responses and transcriptional changes of wheat infected with adapted and non-adapted isolates of Magnaporthe spp. were investigated. Approaches formed beneath attempted penetration sites appeared to prevent colonisation by the non-adapted M. grisea isolate, but were breached by the adapted M. oryzae isolates. Microarray analysis indicated that wheat undergoes extensive transcriptome reprogramming following inoculation with both adapted and non-adapted isolates of Magnaporthe spp. A distinct set of transcripts were induced exclusively in response to the non-adapted M. grisea isolate, while others were induced in response to both adapted and non-adapted isolates. Transcripts induced in common by adapted and non-adapted isolates were differentially regulated in response to M. oryzae and M. grisea isolates over time. Functional analysis of one of these transcripts, WIR1, increased cell-to-cell spread of M. oryzae hyphae, but not penetration efficiency, suggesting a post-penetration resistance role for this gene. This study provides an insight into the development of Magnaporthe spp. on wheat, together with functional characterisation of transcripts differentially expressed during adapted and non-adapted Magnaporthe spp.-wheat interactions.

Resistance among U.S. wheat (Triticum aestivum) cultivars to the wheat pathotype of Magnaporthe oryzae

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Phytopathology 101:S220

Magnaporthe oryzae is the causal agent of blast on several graminaceous plants. The M. oryzae population causing wheat blast has not been found outside South America. U.S. wheat production is at risk to this pathogen if introduced and established. Proactive testing of U.S. wheat cultivars for their reaction to blast and identification of resistance resources is crucial due to the national and global importance of the U.S. wheat industry. In this study, the phenotypic reaction of 72 U.S. wheat cultivars to M. oryzae was determined. Testing of cultivars was performed in a biosecurity level-3 laboratory; all inoculations used the T-25 isolate. Visual assessment of the percentage-killed spikelets or leaf area affected by blast was recorded. To determine if seedling and adult plant severity were correlated, 12 wheat cultivars showing different levels of reaction to blast were inoculated at the head and seedling stage. There was a significant correlation in the reaction to blast at both stages; however, a maximum of 64% of the seedling reactions was explained by the head reactions. Therefore, testing of all 72 cultivars occurred at the head stage. Among cultivars tested at least twice, a continuum in severity to head blast was observed. Cultivars Everest and Karl 92 were highly susceptible with more than 90% disease severity. Cultivar Jagalene was highly resistant with less than 1% severity, as were cultivars Postrock, Overley, Jagger, Jackpot, and Santa Fe with less than 2.5% severity.

An international perspective on wheat blast

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Phytopathology 101:S220

Wheat blast caused by Magnaporthe oryzae has been recorded in Brazil since mid-1990s and occurs in parts of Bolivia, Paraguay and Argentina. It can account for 10 to 100% crop losses. Control of the disease is limited by lack of effective fungicide spray schemes and resistant varieties. A climate similarity approach was used to estimate the risk of wheat blast in other climatic regions. Climatic similarity was derived from the Worldclim database considering the coolest quarter in which wheat is grown in warmer wheat areas, while similarity comparisons with the areas of cultivation in the northern hemisphere were drawn from the warmest quarter of the year. The preliminary analysis revealed areas of risk in parts of Central India, Bangladesh and Ethiopia. Similarity was also identified with areas in the northern hemisphere, Eurasia and North America. However a study using the Homologue software package showed that northern Eurasia and America did not match a year-round climate comparison with areas in South America where wheat blast occurs. Yet a few areas at the border of wheat growing regions in the Indian sub-continent and parts of Africa show a 40–60% similarity with affected areas in South America underlining that risk of wheat blast pathogen survival exists. From the limited knowledge available the survival of wheat blast pathogen in the cool or cold season is unlikely, diminishing the current risk of wheat blast in the northern hemispheric wheat production zones.
A “de novo” origin for the wheat-adapted populations of *Magnaporthe oryzae* in Southern Brazil and levels of gene flow 20 years after the first epidemics

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Phytopathology 101:S221

Divergent lineages of parasites can arise through ecological adaptations. For fungal plant parasites, these ecological adaptations include host shifts or changes in pathogenicity. We compared patterns of genetic differentiation between sympatric and allopatric populations of *Magnaporthe oryzae* causing blast on rice (*Oryza* sp.) worldwide and on wheat (*Triticum* sp.) in Southern Brazil. The primary question was whether wheat and rice support specialized and genetically isolated pathogen populations of the blast pathogen. We also asked what were the current levels of gene flow among geographically distinct wheat-infecting populations across Brazil, based on 12 microsatellite loci. Levels of gene flow between these two host-specific populations were consistent with high population differentiation. Very low historical migration was found between rice- and wheat-infecting populations of *M. oryzae*. However, no subdivision was observed among the wheat-infecting populations. None of the wheat-infecting isolates harbored a functional AVR-Pita, commonly present in rice-infecting isolates. Most of the wheat-infecting isolates carried AVR1-CO39, which is consistent with avirulence on *Oryza* sp. Although the two mating type idiomorphs were detected in few of the Brazilian wheat-infecting populations, MAT-1-1 predominated. We propose that the Brazilian wheat-infecting population was derived “de novo” from an unknown Poaceae-infecting *M. oryzae* population rather than originating from the rice-infecting population.

Risk mapping wheat blast pest in Brazil

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Phytopathology 101:S221

Wheat blast caused by *Magnaporthe grisea* was first reported in 1985 in the state of Paraná and rapidly spread to sub-tropical wheat growing regions of Brazil, Paraguay and Bolivia. It has become a major wheat pathogen in this region. Warm temperatures and high humidity favors disease development. The pathogen infects all above-ground parts of the wheat plant, but spike infection is the main concern. The damage potential is high and can account for 10 to 100% crop losses. Control of the disease is limited by lack of effective fungicide spray schemes and resistant varieties. The pathogen might have evolved from *M. oryzae* isolates from other plant species, but its origin is unknown. The origin of wheat blast epidemics has been elusive, even though evidence shows it is a residue-borne disease. The association of *M. grisea* with grasses provides further evidence for the environmental origin of wheat blast epidemics, as well as an explanation for the sporadic and erratic occurrence of outbreaks. Continued global warming is likely to exacerbate plant diseases problems. Therefore, efforts need to be made for better understanding the potential geographical limits of new diseases like wheat blast. In absence of large amount of quantitative data exploratory simulation models can be used for risk evaluation. A wheat blast simulation model was developed. Model output was used for producing risk maps for a large geographical region.

Epidemiology/Ecology/Environmental Biology of Pathogens

11th I. E. Melhus Graduate Student Symposium: Today's Students Making a Difference in Plant Disease Epidemiology and Disease Management

Climate, weather, and the heterogeneity of Fusarium head blight

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Phytopathology 101:S221

Fusarium head blight (FHB) is a serious yield-limiting and toxin-producing disease of wheat, which is highly variable in time and space. Empirical quantification of the variation in FHB in relation to climate and weather variation will aid in the advancement of disease forecasting. Three analytical approaches were used to address this heterogeneity. First, window-pane analysis was used to investigate the FHB-weather-climate relationship across multiple years and regions in the U.S. and Europe. Moisture- or weather-related variables (e.g., daily relative humidity) were found to be positively correlated with FHB intensity in the U.S., and with disease and/or toxin levels in Europe for multiple window lengths and starting times. Second, cross-spectral analysis was used to show coherency between inter-annual variation in FHB from two locations in the U.S. and global climatic patterns, such as the El Niño-Southern Oscillation. There were significant coherencies at one or more inter-annual time scales (i.e., periods), with the climatic indices for winter or spring leading the FHB series by 2 to 9 years. Third, results from a novel spatial analysis of survey data using generalized linear mixed models confirmed that FHB incidence is also heterogeneous on multiple spatial scales, with variation larger at the county or field scale relative to the within-field (sampling-unit) scale. This likely resulted, in part, from environmental variation among counties and fields.

Spatial distribution of brown rot symptoms and fine-scale genetic structure of populations of *Monilinia* spp. within and among stone fruit tree canopies

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Spatial patterns and dynamics of plant disease and pathogen populations can provide important insights into epidemiological processes such as sources of inoculum, mechanisms of inoculum dissemination, and reproductive strategies of the pathogen population. For brown rot of pome and stone fruits, caused by *Monilinia* spp., the spatial arrangement of infected trees in the orchard has been examined, but spatial patterns of and relationships among different symptom types (such as blighted blossoms, shoot blight, and twig cankers) within individual trees have not been analyzed previously. We mapped coordinates of all brown rot symptom types caused by *Monilinia laxa* in eight individual sour cherry tree canopies using a digital digitizer. Three-dimensional nearest-neighbor analysis showed that symptomatic canopy elements were significantly aggregated compared with complete spatial randomness in each case, but there was no evidence that one symptom type was more aggregated than another. Pairwise association analyses showed that nearly all trees had significant spatial associations between one or more symptom types, and that previous year’s twig cankers may play an important role in affecting the spatial pattern of current year’s symptoms. Second-order spatial statistical analyses are being developed for more detailed quantitative assessment of patterns of diseased elements and of fine-scale genetic structure of the pathogen population within the tree canopy.

Effects of temperature and wetness duration on the sporulation rate of *Phomopsis viticola* on infected grape canes

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In 2008, research was initiated to examine effects of temperature (T) and wetness duration (WD) on the sporulation rate of *Phomopsis viticola* on infected grape canes and to determine effects of interrupted wetness duration (IWD) on sporulation (S). To determine effects of T and WD on S, a split-plot design was used, with T (5, 12, 15, 18, 20, 22, 25, 28, and 35°C) as the whole-plot and WD (11, 23, 35, 47, and 71 h) as the sub-plot. Linear and nonlinear models were fit to the data. Goodness of fit was based on residuals, pseudo-$R^2$, -2 log likelihood, parameter effects nonlinearity, correlation of parameter estimates, and other statistics. Lower and upper limits of S were found to be 5 and 35°C, respectively. Optimum S was near 22°C, and S increased with increasing WD. Of the examined models, Analytis’ Beta model fit the data best. To determine effects of wetness interruption, a split-plot was used, with T (12, 15, and 20°C) as the whole-plot and IWD (0, 2, 4, 8, 12, and 24 h) as the sub-plot. Generally, S declined with increasing WD. Of the examined models, Analytis’ Beta model fit the data best. Vol. 101, No. 6 (Supplement), 2011
After a ten year eradication program, *Phytophthora palmivora* eradication programs were declared eradicated in Pennsylvania (PA) in 2009. Despite imposing a similar program in Ontario (ON) in 2000, PPV has not yet been eradicated there. The U.S. and Canadian eradication programs differ in a number of key regulatory features, including no. leaves collected per tree, ELISA test kit used, and tree removal protocols. The objective of this research, therefore, was to quantify the effects of the Canadian and U.S. eradication programs on the PPV epidemics at several spatial scales. Spatial dependence ranged from 0.7 to 4.3 km for PPV-positive blocks in PA, while in ON spatial dependence ranged from 1 to 25 km. The agreement between the U.S. and Canadian ELISA kits was significant at the leaf and scaffold scale, but not significant at the tree scale. A simulation model was developed to compare the detection efficiency of the U.S. and Canadian sampling protocols. Stratified sampling by scaffold did not significantly ($P < 0.05$) improve detection efficiency compared to a random sampling design. Finally, sample sizes requiring only one PPV-positive leaf per bulk sample were optimal. The data generated in this study should help to improve the sampling and detection efficiencies for PPV eradication programs in both countries.

### International Mycotoxin Issues in a Changing World

#### Potential strategies for preventing recurrent aflatoxicosis outbreaks in Kenya

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Phytopathology 101:S222

Recurrent outbreaks of aflatoxicosis in Kenya underscore the need for proactive preventive strategies. However, the incidence of contamination and levels of aflatoxin in food commodities can vary tremendously, and staple foods in Kenya are mostly homegrown or traded in informal markets, which hinders effective monitoring. Working with peanuts, we have identified factors associated with the risk of contamination. The incidence of peanut contamination was significantly associated with the agro-ecological zones predominant in the district of sample origin ($\chi^2 = 9.18; P = 0.002$), and whether the peanut cultivars were improved or local landraces ($\chi^2 = 4.27; P = 0.039$). Microbial analysis revealed that contamination with *Aspergillus flavus* S-strain was the most important factor determining the likelihood of a sample exceeding Kenya’s aflatoxin regulatory limit of 10 µg/kg ($P < 0.001$). Membership to Producer Marketing Groups ($\chi^2 = 6.01; P = 0.014$), which train farmers on tactics that reduce aflatoxin contamination, and grading peanuts ($\chi^2 = 4.75; P = 0.029$) were negatively associated with the incidence of samples contaminated with the *A. flavus* S-strain. Crop rotation was correlated with reduced incidence of the B but not the G aflatoxins while cultivar improvement was negatively correlated with the incidence of the G aflatoxins. Identifying ecological, agronomical and/or socio-cultural factors linked to maize contamination will enable development of strategies to reduce the risk of aflatoxicosis.

#### Risk index assessment of aflatoxin contamination of peanut

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Phytopathology 101:S222

Predictive models are frequently cited as aids for making plant disease management decisions. However, such systems may not be implemented due to their dependence on unreliable or limited weather forecasts. Alternatively, a Risk Index approach overcomes some of these limitations and can account for other factors that contribute to disease development. Such a risk index has been developed for aflatoxin contamination in peanuts. This risk index accumulates values as the season progresses and greater values reflect a higher probability of aflatoxin contamination. Risk factors include nematode and insect pests of peanut, insufficient soil calcium, and, most critically, hot and dry conditions prior to harvest which accounts for 50 points of the 120 maximum in this index. Over several growing seasons, aflatoxin contamination of peanuts has been monitored in growers’ fields across Alabama. Sums of risk values have ranged from 20 to 92. Peanuts from fields with index values $< 20$ ($n = 59$) had a 97% probability of having low aflatoxin contamination ($< 20$ ppb) while those from fields with index values $> 74$ ($n = 15$) had a 66% probability of high contamination ($> 20$ ppb). This approach is simplistic but readily implemented; it is also imperfect. In general, little can be done to manage aflatoxins in rainfall peanuts; however, implementation of this risk index might affect harvest decisions.

#### Evaluating human exposure to fumonisins in Guatemala and its possible role as a contributing factor to neural tube defects

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Phytopathology 101:S222

Fumonisins (FB) is an inhibitor of sphingolipid (SL) biosynthesis and folate transport and can induce neural tube defects (NTD) in mice. NTD incidence is high in countries where maize is a dietary staple and FB exposure is likely. In Guatemala FB in maize has been documented and a preliminary exposure assessment has been conducted. Research in Guatemala has identified communities where FB in maize is frequently high and exposure confirmed in preliminary studies using urinary FB as an exposure marker. Currently we are evaluating information from studies in humans and animals to assess and validate the use of SL and FB biomarkers in women known to consume large amounts of maize potentially contaminated with FB in Guatemala. The project is analyzing for FB in maize samples from local markets and collecting urine and blood spots to validate biomarkers in the high and low exposure communities. The protocols for the human studies are IRB approved. In addition to the human studies, studies in mice will determine the dose-response relationships in urine FB and SL metabolites and genetic markers in populations that correlate with increased risk for NTD in n. The information from the proposed studies will form the basis for the design of prospective and retrospective epidemiological studies to identify women at high risk for NTD (Supported in part by the PHS-NIH Eunice Kennedy Shriver National Institute of Child Health & Human Development and NIH Office of the Director IRC4HD067971-01).

#### Mycotoxins in Asia and other countries—2009–2010

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Phytopathology 101:S222

Samples of various commodities were collected from various countries throughout the world including North America, South America, Northern, Central and Southern Europe, The Middle East, Northern, Southeastern and Southern Africa and Oceania. A total of 6076 samples including corn (maize), soybean meal, wheat/bran, corn gluten meal, rice/bran, distiller’s dried grain with solubles, prepared feed, straw, silage and barley. These samples were analyzed for aflatoxins, zearalenone, deoxynivalenol, fumonisins, and ochratoxin A using either HPLC or ELISA tests (USA and Europe only) at laboratories including Romer Labs Singapore, Romer Labs Austria, Romer Labs USA and Simittec Brazil. The global occurrence of mycotoxins for the 2 years established that aflatoxins were found from a relatively low (8%) number of samples in North and Central Europe while considerably higher percentages were found in Southern Europe and Asia, especially in the southern parts of the latter. Generally, all of the mycotoxins were found in a higher percentage of samples in all of Asia than in Europe, Middle East, North and South America and Oceania. Globally, deoxynivalenol and fumonisins were the most frequently found mycotoxins from NTD risk countries. Species and type of mycotoxin and overall worldwide distribution of these mycotoxins (including co-occurrence) will be discussed.

#### Pathogenesis by mycotoxigenic fungi: The tipping points

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Phytopathology 101:S222

Increased climate variability associated with climate change is predicted to increase the magnitude and distribution of pre-harvest contamination with mycotoxins. Mycotoxin contamination of developing grain is highly dependent of environmental conditions. As an example, the major factors contributing to high concentrations of aflatoxins are high temperatures and dry conditions favor growth, conidiation, and dispersal of *Aspergillus flavus* and impair growth, development, and the expression of resistance in maize. In addition, these conditions appear to impact the population structure of the fungus. Less clear is the effect climate change may
have on the production of other secondary metabolites, many of which have not been characterized and some of which may be toxic. Aflatoxin has 55 predicted clusters for secondary metabolism, each of which likely produces multiple toxins that have not been described. A transcriptional analysis of these clusters showed some to follow the pattern of aflatoxin biosynthesis, some to be favored by different environmental conditions and others to be silent under the 28 conditions examined. Conditions that favor aflatoxin biosynthesis for example, are conducive for the biosynthesis of cyclopiazonic acid (CPA), but not aflatoxin. We may be underestimating the impact of climate change on mycotoxin contamination.

**Why Care About Crop Loss? Impacts on Science, Production, and Society**

Why do we care about crop losses? S. SAVARY (1), E. Duveiller (2), J. Aubertot (3)

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Phytopathology 101:S223

Crop losses caused by pests have particularly important consequences in highly populated areas. This is because of their impact on food security, the large population affected, and difficulty in implementing mechanisms for dealing with food shortage. This presentation will focus on South and South-East Asia and rice, which is the major staple food in this region. Approaches to estimate crop losses due to rice pests at the regional scale will be described and applied to determine the relative importance of rice pests in terms of crop losses, depending on the biophysical and socio-economic context of production. Pest management strategies derived from these findings will be discussed. Perspectives related to opportunities and bottlenecks in assessing crop losses and the use of crop loss information will be discussed in terms of science, technology, and socio-economic environments.

**Impact of crop loss in the United States**

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Phytopathology 101:S223

Plant diseases were first studied because of a need to understand yield losses associated with them. There was a need to know the impact on crops due to the fear of not providing ample quantities of food to feed the population. Although this was a worldwide phenomenon, it was certainly true in the developing years of the U.S. In more modern times the fear of providing enough food to the U.S. population has lessened but the need to understand yield losses is just as important as ever. How are we to determine research and extension priorities or how much to spend on them when we do not have reliable yield loss data? How do government authorities determine strategies for storage, movement of excesses in one area to areas of production shortfalls and use when we do not have reliable yield loss data to determine areas of need? How do governments and industries determine import and export needs without reliable yield loss data? There are many great examples of yield loss measurements in the U.S. for priority determination but as a whole we lack a concerted effort to have more than the “educated guess.” The loss assessment area is wide open for plant pathologists and the need for this information is growing.

**Better Use of Entomopathogenic Microbes in IPM**

A bioprotection strategy for greater integration of beneficial microbes into IPM

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Phytopathology 101:S223

Microbial pathogens and antagonists (MPA) are an often overlooked component of pest management for integration into IPM. Under a bioprotection strategy, which aims to maximise the impacts of biological control, MPA take a greater role. MPA in situ are an important component of conservation biological control as they undermine pest population vigour. In situations where MPA populations are low, they can be added to establish in the pest’s environment and initiate epizootics of disease to prevent pest build up, and
finally, where high, healthy pest populations are out of control, MPA can provide a curative effect when applied as microbial biopesticides to rapidly reduce pest attack. Successful implementation of bioprotection relies on new tools for determining pest health status and predicting pest outbreaks, which can be provided by molecular biology and population modelling. Examples will be given from pasture, plantation and field crops where bacterial, fungal and viral agents have been used. By placing emphasis on the microbial component in pest management, bioprotection leads to enhanced IPM.

Release of *Beauveria bassiana* insecticide does not cause silkworm white muscardine

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White muscardine caused by *Beauveria bassiana* is an important limiting factor of sericulture. 124 *B. bassiana* isolates were collected from silkworm cadavers, rearing rooms and appliances, surrounding mulberry orchards, pine plantations and cropland in Qianshan, Southwest Anhui Province and Jingxian, South Anhui, China. Together with the mass production strain, the isolates were analyzed for population genetic structure by ISSR markers to trace the origin and spreading track of the muscardine. The results showed that the two populations were heterogeneous. The muscardine subpopulation of Qishan was polyphyletic, while those of Jingxian were monophyletic. The later and the predominant subpopulation of the former were characterized typically by enzootic nature, but the non predominant muscardine subpopulation of Qianshan might be able to spread among some field alternate hosts. The groups prevailing in pine caterpillar populations surrounding pine plantations, the production strain was not associated to the silkworm muscardine. A bioassay revealed that LC25, LD25 and LT50 caused by the isolate from infected pine caterpillars were 1327, 1378 and 1.5 times, as high as a typical pathogenic isolate from silkworms, respectively. Based on obvious host specificity of the isolates from the caterpillars, the use of *B. bassiana* insecticide developed from these isolates against the caterpillars is comparatively safe to sericulture.

Microbial control in Brazil

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The development of microbial agents to control insect pests in Brazil has resulted in significant programs since 1964, leading to their wide use in the country especially entomopathogenic fungi, mainly *Metarhizium anisopliae* applied against sugar cane pests, although research with fungi started as early as 1923. *Bacteria*, mainly *Bacillus thuringiensis* have been available since the earliest 70’s, based on imported products but more recently a few private Brazilian companies have developed commercial Bt formulations either for agricultural pests or vectors of human diseases, such as dengue. The main activities with nematodes have been initiated recently, as far as commercial production to control insects in sugar cane, coffee as well as protected crops. Regarding viruses, great achievements were obtained with the *Nucleopolyhedrovirus* (NPVs) have efficacy against insecticide resistant *Spodoptera exigua* in shallot plantations in Taiwan. Again, the action of these entomopathogenic organisms is combined with other biopesticides (e.g. neem oil) to obtain a broader control of *Maruca vitrata*, two key cowpea pests in Africa. A strain of *M. anisopliae* is currently being commercialized against flower thrips in East Africa, while the *Maruca vitrata* Multiple Nucleopolyhedrovirus (MNPV) is being developed into a commercially available biopesticide in Taiwan. Again, the action of these entomopathogenic organisms is combined with other biopesticides (e.g. neem oil) to obtain a broader control of *Maruca* pests in a sustainable management approach.

Microbial control in Australian cropping systems

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Resistance to chemical insecticides led to a crisis in Australian broadacre cropping in the late 1990s. This in turn led to the adoption of an integrated management strategy across mainstream cotton and grain industries, which included the use of biopesticides. Biopesticides based on *Nucleopolyhedrovirus* (NPVs) have efficacy against insecticide resistant *Helicoverpa* spp. and low impact on natural enemies. They are widely to manage Heliothine pests specifically to manage insecticide resistance and to reduce further pest outbreaks by maintaining beneficial insects. A number of commercial NPV biopesticides are now registered. High quality, product availability and viable pricing created a successful market in a broad range of commercial crops including sorghum, cotton, sweetcorn and lettuce. Further research in targeting, timing and formulation led to improved field efficacy. In grain sorghum, 95% of the national sprayed crop area is treated with NPV biopesticides. The success of NPVs has increased grower confidence in biopesticides as part of an integrated strategy and led to a demand for similar ‘soft’ options against emerging sucking pests. Continuing research has resulted in development of prototype commercial insecticides based on fungal pathogens against a broad range of pests.

Making use of microbes in pasture bioprotection

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Pastures in New Zealand have traditionally been managed as low-input systems, with minimal use of chemical pesticides for insect control. Pest management has relied on developing resilient pasture swards and applying insecticides, such as seed treatments, with grazing animals selection of pasture species with cultivated forage crops and breeding insect-tolerant pasture plants. Recent intensification of pastoral farming has required additional tools, such as microbial pest control agents. The most well-known of these is *Serratia entomophila*, a bacterium that causes disease in the New Zealand grass grub (*Costelytra zealandica*). In situ management practices, such as minimising cultivation, have been used to maintain levels of this microbe in pasture. In addition, it has been developed as a biopesticide for inundative control, firstly as a liquid formulation (*Invade®*) and more recently as a drillable granule (*Bioshield™*). Threshold populations of larvae and pheromone traps to detect beetles are tools that are being developed for integrated management of grass grub. Currently other microbes are being tested as biopesticides for other pasture pests, such as *Yersinia entomophaga* against *Wiseana* spp. and *Beauveria bronniarii* against *Pyronota* spp.

Promising new biopesticides for use in microbial control of major pests in African cropping systems

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The development of biopesticides based on entomopathogenic organisms was spearheaded in Africa by the LUBILOSA project, which successfully developed a selected strain of *Metarhizium anisopliae* into a commercially viable product, Green Muscle™, used for the control of hopper bands of locusts and sahelian grasshoppers. Spin off research from this project included the search for novel entomopathogenic organisms against the variegated grasshopper *Zonocerus variegatus*, diamondback moth *Plutella xylostella*, and termites. Also, the synergistic effects of low doses of conventional insecticides sprayed together with biopesticides were assessed in the context of an IPM approach. At the same time, an ecological model used to investigate the interactions between Green Muscle™ and predation revealed that hoppers treated with biopesticides get slower with time and become easy prey for birds. Collaborative studies with icipe and AVRDC have investigated the possibility of developing biopesticides against flower thrips and the pod borer *Maruca vitrata*, two key cowpea pests in Africa. A strain of *M. anisopliae* is currently being commercialized against flower thrips in East Africa, while the *Maruca vitrata* Multiple Nucleopolyhedrovirus (MNPV) is being developed into a commercially available biopesticide in Taiwan. Again, the action of these entomopathogenic organisms is combined with other biopesticides (e.g. neem oil) to obtain a broader control of cowpea pests in a sustainable management approach.

Microbial control of arthropod pests, a key component of IPM programs in Indonesia

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Most farmers in Indonesia are low income farmers with very limited land ownership. This is especially true for those that produce vegetable crops. The main obstacle in their farming is the shortage of funds. However, when pest problems occur, they continue to use high amounts of expensive chemical pesticides. A wide variety of insect pathogens are available in Indonesia that can serve as effective alternatives to pesticides. A nucleopolyhedrovirus has been used successfully to control Spodoptera exigua in shallot plantations in Central Java. Other examples include the use of *Beauveria bassiana* to control *Helicoverpa* spp. on cotton, *Metarhizium anisopliae* and a nonoccluded Baculovirus to control *Oryctes rhinoceros*, a nematode, *Steinernema sp.* to control thrips on chili pepper, and a microporidian, *Nosema* sp., to control locusts. As part of the IPM CRSP Project in Indonesia, Bogor Agricultural
Crop Health Management for Food Safety and Agroecosystem Health in Developing Countries

Disseminating good agricultural practices in vegetable production for better human and agroecosystem health

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Phytopathology 101:S225

For smallholder farmers, sustaining the productivity of the limited land they own depends on the health and maintenance of the agroecosystem in their plots. Vegetables are important income and nutrient sources for smallholder farming households in developing countries and can be cultivated in diverse agroecosystems with various levels of inputs. Numerous technologies have been developed to improve vegetable productivity; the challenge is to introduce suitable, agroecosystem-enhancing technologies that have maximum potential to be adopted by local communities. Technology dissemination for impact generation starts from understanding local production constraints, cultures, and socioeconomic status. Certain technologies with good adoption potential are then selected. Participatory trials for adapting selected technologies are designed and conducted with local stakeholders. The adapted technologies are then disseminated using effective strategies designed with local partners. AVRDC – The World Vegetable Center aims to build local capacity by applying the research and development cycle on a regular basis. This is the key to increasing the resilience of smallholder vegetable farmers against climate change. The Center recently disseminated integrated crop management technologies in Aceh, Indonesia after the 2004 tsunami and in the Solomon Islands. The lessons learned will be presented.

Advances in integrated aflatoxin management in Africa

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Phytopathology 101:S225

Aflatoxin contamination is widely distributed across sub-Saharan Africa. Crop aflatoxin levels must be significantly reduced to improve human and animal health and increase the marketability of agricultural products. CGIAR recognizes aflatoxins as an important constraint for improving the human condition through agriculture. Various CGIAR strategies are pursued in an integrated research-for-development pathway to improve aflatoxin management from field to fork. Strategies include problem definition (e.g., information on prevalence, impact on health and trade, climate change effects, biology of causal agents), technology development (e.g., biocontrol and resistant cultivars), technology adaptation (e.g., good harvest and storage practices), technology testing and integration (e.g., combining different methods with user feedbacks), technology dissemination (e.g., farmers’ field schools) and advocacy (e.g., awareness, education, policy support, and networking). One of the major advances in aflatoxin management in Africa is the adaptation of biological control using native atoxicogenic strains of Aspergillus flavus. Biological control is on the verge of large-scale adoption in Nigeria and development is progressing in Kenya, Senegal and Burkina Faso. We continue to address issues related to awareness, advocacy, education and regulatory support that are the foundations on which aflatoxin management efforts are institutionalized. Partnership between CGIAR centers and with other institutions is the key to this approach.

Seeing the unseen - Improving agroecosystem health through sustainable nematode management in smallholder systems

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Nematode pests are regularly overlooked and misdiagnosed, in part, due to their subterranean and cryptic habit. Symptoms of nematode damage may be observed on both aerial and root parts. Root necrosis, stunting and galling are typical, reducing efficient access to available water and nutrients. Affected plants may present symptoms of nutrient deficiency and water stress. This lack of specific symptoms complicates diagnosis. The global scarcity of nematode expertise further compounds identification of nematode problems. Under resource poor conditions, the situation is exacerbated by the need to intensify production to feed burgeoning populations, especially those in urban areas. Consequently, the limited focus on nematode management needs redress. In smallholder production systems, nematodes gradually manifest, resulting in substantial production losses. In intensified smallholder systems, such as peri-urban vegetable gardens, pesticide use has become a common practice which often does not account for an underlying nematode problem, leading to misuse of synthetic pesticides. The limited availability, inconsistent quality and perceived high cost of pesticides, in addition to a limited awareness of the most adequate pesticides for the specific situation, creates further problems. Innovative IPM options for nematode management developed in a multi-discipline approach will become increasingly necessary to overcome production losses.
Integrated management of food legume diseases for sustainable rainfed agroecosystem

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Phytopathology 101:S226

About 65% of the 522 million people live in rain-fed agro-ecosystem in South Asia and severely affected by mal nutrition leading to poor physical productivity and to higher rates of non-communicable diseases. Food legumes [chickpea, lentil, field pea, pigeon pea, blackgram, mungbean, cowpea and soybean are the major source of inexpensive protein for rural poor in rain-fed South Asia. For example a grain of protein from grain legumes varied from 0.1- 0.34 rupee in India compared to 0.86 rupees from meat. Inclusion and expansion of food legumes in rain-fed cropping systems not only provide nutrition to poor, as well as increases the productivity and sustainability of rainfed smallholder farmers. However, evidences revealed that food legumes are prone to root rots, wilt and foliar diseases caused by fungi, bacteria and viruses; incurring crop losses up to 100% in susceptible cultivars. This paper discusses the opportunities for greater integration of disease management of food legumes into their production agronomy in the rain-fed cropping systems for their profitable popularization. The integrated management of food legumes involves host plant resistance, agronomic practices, judicious use of fungicides, pesticides for vector control, and biopesticides for pathogen control, risk forecasting that operate on different aspects of the disease etiology, such that they complement each other and can be applied together in farmers’ fields collectively to provide farmers with maximum economic return.

Role of insect-resistant transgenic crops for pest management and their impact on environment and food safety

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IPM Program for Vegetable Crops in the Tropics and Opportunities for IPM Graduates

IPM program for vegetable crops in Central Asia

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Phytopathology 101:S226

Michigan State University, University of California-Davis and Kansas State University in collaboration with the CGIAR/ICARDA-Project Facilitation Unit are implementing an ecologically-based regional IPM program in Central Asia to enhance food security in the region. This regional IPM program is funded by the USAID funded IPM CRSP program managed by the Virginia Tech University. The focus of this regional program is to develop IPM packages for two important vegetable crops, Tomato and Potato. The IPM packages for these two crops are developed through applied research and demonstration sites in host countries in Central Asia including Uzbekistan, Kyrgyzstan and Tajikistan. The project is enhancing institutional capacity building through graduate training, workshops, short courses, joint publications, and outreach programs. In developing and delivering IPM packages, the project also addresses cross-cutting issues such as gender and socio-economic aspects.

IPM tactics for vegetable crops in Indonesia

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Phytopathology 101:S226

The most common vegetable crops cultivated in Indonesia are cabbage/broccoli, tomatoes, chilli pepper, shallot/onion, and yardlong bean. Numerous

Harvesting Agro-Ecosystem Resilience in water limited wheat based cropping systems–a major challenge for food security in West Asia and North Africa

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Phytopathology 101:S226

Wheat is a key to food security in much of West Asia and North Africa (WANA). The rainfed wheat is mostly grown under a conventional wheat fallow system and exposed to climatic extremes including frequent droughts and heat stress, making the resulting the soil health status (physical, chemical and biological) is far from sustainable. Biotic soil borne pathogens (SBPs) including Cereal Nematodes (cereal cyst – Heterodera spp. and root lesion – Pratylenchus spp.) and the root rotting fungal complex (Fusarium spp. and Bipolaris sorokinana) frequently occur together in cereal soils reducing the ability of the roots to absorb water and nutrient. These abiotic and biotic constraints often occur in combination and interact synergistically to reduce wheat productivity. Although SBPs are widespread across WANA, they often unrecognised by Wheat Improvement scientists as they are soil borne and are associated with non-specific about ground systems easily confused with other systems. CIMMYT International working with Turkey, and sister centres ICARDA along with many National Program partners in WANA have identified components for use in integrated control packages to harness agroecosystem resilience for these wheat systems. These includes germplasm enhancement for multiple SBP resistance and/or tolerance; combined with most appropriate conservation agricultural practices (reduced tillage, use of rotation and/or residue retention); and seed treatments to enhance plant health. Capacity building is one of the key mechanisms to enable further research and uptake of these components.
insects and diseases are known to reduce vegetable production, and IPM tactics have been developed and practiced to overcome these problems. Various biological control agents are being implemented. These include application of *Trichoderma harzianum* to control club root disease on crucifers and other soil-borne vegetable diseases. Dipping seedlings in *Bacillus subtilis* and *Pseudomonas fluorescens* reduced infection by fungi and screened-seed beds suppressed virus infection on tomatoes and chili pepper. Farmer-level production and use of the nucleopolyhedrovirus (SnPV) to control Spodoptera exigua on shallot is an effective tactic. The naturally occurring parasitoid, *Hemitarsus varicornis*, and a predatory fly, *Coenosia humilis*, control populations of *Liriomyza huidobrensis*, when broad spectrum insecticides are avoided. Some physical/mechanical controls include fine-mesh netting and use of black-light traps in shallot. Hand-picking larval clusters and spot treatments with *Bacillus thuringiensis*, effectively controlled the cluster caterpillar, *Crocidolomia pavonana*. Botanical extracts of *Tephrosia vogelii* are used to control *Neotoxicotera formosana* on green onion and *Phyllotreta striolata* on crucifers. Cultural methods employed include crop rotation, soil liming, plastic mulching, and reduction of infection sources.

**IPM technologies developed for vegetable crops in the Philippines**

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Phytopathology 101:S227

Four yield-enhancing, cost-reducing, and environment-friendly technologies - VAM (Vesicular Arbuscular Mycorrhizae), *Trichoderma sp.*, NPV (nuclear polyhedrosis virus), and sex pheromone traps - were developed and promoted to improve farmer competitiveness, minimize environmental risks, and ensure food security. VAM, a beneficial fungus, acts as a biofertilizer and biological control agent (BCA) against vegetable diseases. *Trichoderma sp.*, another beneficial fungus, functions as a biological fungicide. Impact assessments of these technologies showed a 23% increase in yield, 43% reduction in cost of fungicides, and 19% decrease on fertilizer cost, resulting in a 167.58% increase in adopters' net income over the non-adopters. The use of NPV against cutworms and armyworms resulted in a net incremental benefit of $136,000/ha compared to $65,000/ha using insecticide. The use of sex pheromones, a pest monitoring tool that helps in deciding the proper timing of intervention, resulted in a 70–90% reduction in insecticide application without reducing yield. Assessments further showed that the increase in net income contributed to the increase in farmer’s capacity to purchase basic needs and accumulate assets. Adopters were also able to establish new social networks and reduce the risk of pesticide poisoning in their households. The four technologies now form part of a package of IPM technology recommendations for vegetable production in the Philippines.

**FAO at Work: Case studies of vegetable Integrated Pest Management and farmer education in Asia**

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Phytopathology 101:S227

Pesticide use in vegetable production among smallholder farmers in Asia remains unnecessary high. Concerns over food safety, farmer health and environmental pollution caused by indiscriminate use of pesticides call for promotion and employment of more sustainable crop production and protection strategies. During the last decade, FAO has been working intensively with Asian governments, civil society organizations and the private sector to develop robust Integrated Pest Management strategies for a range of economically-important vegetable crops during the last decade. Case studies of successfully employed vegetable IPM farmer education initiatives, using the Farmers Field School approach, will be detailed in this paper.

**IPM packages for vegetable crops in India**

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Phytopathology 101:S227

In Indian sub-continent, vegetable crop productivity is limited due to damage caused by many insect pests and diseases besides nematodes. Excessive use of synthetic pesticides resulted in development of resistant insects, adverse effects on human health and the degradation of environment. Sustained efforts were taken to integrate locally adapted farmer friendly both conventional and biorational methods of pest management through USAID funded IPM-CRSP project at TNAU, INDIA. The IPM packages were developed and validated through farmers’ participatory approach in onion, eggplant, okra and tomato. In these vegetable crops, an IPM package of using different components like seed treatment, nursery application and soil application in main field with *Trichoderma viride* and/or *Pseudomonas fluorescens*, application of neem cake, selection of virus disease free seedlings for planting, roguing out of virus infected plants, growing marigold as a border crop, growing trap/barrier crops, setting up *Helicoverpa* / *Earias* / *Spodoptera* / *Lecinoides* pheromone traps, release of *Trichogramma chilonis*, installation of yellow sticky traps, spraying neem formulations / neem seed kernel extract and need based application of nematicide/insecticides/fungicide was found to yield significant reduction in pest problems and increased income to the farmers. The BC ratio varied from 1.86:1 to 4.89:1 in different vegetable crops. The IPM package development is in progress in hot pepper, cabbage and cauliflower.

**Technology transfer of vegetable IPM packages in India**

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Phytopathology 101:S227

IPM demonstrations were carried out in U.P., A.P. and Karnataka under USAID IPMCRSP program. Moretha 80 trials have been conducted covering total area of 41.5 acres. Technologies for seed and seedling treatment with *Trichoderma viride* and *Pseudomonas fluorescens*, use of pheromone traps, yellow sticky traps, neem, NPV’s and *T. viride* spray, mulching, roughing, field scouting, shoot clipping and need based pesticide application were transferred to the farmers in these villages. The training on IPM through More than 80 trials These interventions have resulted in good quality of vegetables, lower cost of production, more profits than conventional pest management, 2 to 3 times more price for IPM produce, better taste, and 50 to 60 per cent reduction in pesticide use. The higher income-used for children education, housing etc.

**IPM: Changing the Vegetable Pest Management System in Bangladesh**

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Phytopathology 101:S227

In Bangladesh, millions of farmers earn their living by growing vegetable crops almost throughout the year. To protect the crops from pest damages the farmers incur more than 30% of the cultivation costs to purchasing pesticides only. Implementation of IPM technologies impacted greatly in changing the mindset of the farmers from resorting to pesticides to using IPM practices. Nine IPM technologies that are now available for about 20 kinds of major vegetable crops include pest and disease resistant varieties, grafting technique, use of bio-control agents, use of soil amendments with poultry refuse, mustard oil-cake or *Trichoderma*-based compost, IPM approach to control cabbage and cauliflower pests, and weed management at critical crop stage. The technologies were highly effective to control 30-95% pests and diseases, reduced pesticide use by 50-90% and increase economic returns by 200-300%. Farmers are now producing pesticide-free vegetables in different areas of about 20 districts of the country. Preliminary estimates show that the farmers saved about US$300,715 by avoiding pesticide use of about 42,000 liters through adopting IPM practice of pheromone baiting in cucurbit (gourd) crops during 2007–2010, and the practice fetched them US$3,007,000 from an additional production of 21,050 tons of cucurbit (gourds). A silent revolution is thus going on at the farm level as the words of IPM success stories are transcending the borders of each success area. The IPM CRSP activities, launched in 1998 with the Bangladesh Agricultural Research Institute (BARI) as the lead collaborator, are continuing to develop and implement IPM practices so as to reach the larger farming communities of the country.

**IPM packages developed for high-value horticultural crops in Latin America and the Caribbean**

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Phytopathology 101:S227

The IPM CRSP has been working in Latin America and the Caribbean region for more than 15 years. Research conducted in collaboration between host country and U.S.-based scientists has led to numerous IPM practices for the control of pests and diseases. As the project matures, its focus has evolved from identifying practices to address specific pest problems to development of IPM packages to solve crop-specific pest complexes. Packages for potatoes and Andean Fruits in Ecuador are now being disseminated and the CRSP is in the final stages of development of packages for peppers, tomatoes and cucurbits in Central America. This presentation will discuss some of the features of these packages, experiences in and obstacles to IPM package development, and potential for cross-country learning about IPM from a package-based global research project.
Phytopathology 101:S228

L. V. MADDEN (1)
Sampling for detecting fungicide resistance

Threshold sensitivity values defined as “resistant” are best supported by fungicides for which quantitative resistance occurs, claims for e.g., blockage of an alternative respiration pathway that fungi might otherwise preventatively as opposed to the recommended curative role. In a bid to chemical pesticides with the majority of the growers using the pesticides D. G. PFEIFFER (1), D. E. Mullins (1), R. L. Gilbertson (2), C. C. Brewster (1) Makerere University, Kampala, UGANDA; (8) Crops Research Institute, Kumasi, GHANA Phytopathology 101:S228

The West African regional project of IPM CRSP includes Ghana, Mali and Senegal, and focuses on tomato, potato and cabbages, aiming to develop IPM packages addressing all of the major pests with which local farmers must contend and to determine appropriate technologies. Cooperators in each of the participating countries attended a 2-day planning workshop in Bamako Mali in order to prioritize pest insects, pathogens and weeds. On tomato, a major pathogen is Ralstonia, causing bacterial wilt, and Alternaria solani, causing early blight. Cultivars were assessed for resistance; proper seed selection and treatment are considered. In Mali, a 2-month host free period was successful for managing whitefly-borne viruses and is tried in Ghana. In addition to whiteflies, Helicoverpa armigera is a major tomato pest. Yellow sticky traps and neem are evaluated. Nineteen weed species were found in a survey of tomato plantings. On potato, potato tuber worm, bacterial wilt and rots of tubers are most important. In cabbage, Plutella xylostella, Helliula undalis, Crocodolomyia pavonana, Helicoverpa and Spodoptera exempta are the most important insect pests. A cabbage IPM package may include biological control intercropping with tomato, botanical insecticides, microbial insecticides, row covers, synthetic insecticides and improved monitoring. The use of non-chemical tools will delay development of insecticide resistance, and minimize use of highly disruptive classes of insecticides.

IPM programs for vegetable crops in the tropics and opportunities for IPM graduates packages for horticultural crops in Uganda

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Phytopathology 101:S228

Of all the horticultural crops in Uganda, tomato is the heaviest consumer of chemical pesticides with the majority of the growers using the pesticides preventatively as opposed to the recommended curative role. In a bid to minimize reliance on chemical pesticides on tomato, research on development and dissemination of IPM technologies applicable on small holdings has been ongoing with funding from IPM CRSP. The technologies that have been found to be effective are mulching, staking, grafting, resistant germplasm; combined with a reduced pesticide spray schedule. Some of the technologies have already gained popularity with farming communities. The project is also branching into developing innovative soil borne diseases’ management using antagonistic micro organisms – a case in point being the on going research on Arbuscular michorrhiza Fungi for nutrient uptake promotion and disease management. Emerging challenges include the unprecedented increase in occurrence of viruses on tomato. The project is responding to the threat by acquiring and testing germplasm from AVRDC as well vector management strategies. IPM CRSP also supports graduate training with two MSC students working on developing IPM technologies on tomato.

Opportunities for graduates of IPM and related areas in international agriculture

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Phytopathology 101:S228

Increasing agricultural productivity is key to improving the livelihood of farm families, alleviating food insecurity and improving prospects for economic growth in developing countries. Plant diseases, insect pests and weeds significantly reduce crop productivity, and misuse of pesticides is very common. While richer nations have made investments in developing countries for decades, their focus has returned to agricultural development as a means of poverty reduction. This has resulted in a demand for expertise in disciplines that directly impact food security. Graduates of IPM and related programs can contribute to these efforts as volunteers or through public or private sector employment. For example, International Agricultural Research Centers provide employment opportunities in modern laboratories that rival those in the developed world. Private sector development organizations seek a wide range of technical expertise for short- and long-term projects. Land Grant Universities have a long history of participation in agricultural development. Programs such as the USAID-funded Collaborative Research Support Programs, particularly the IPM CRSP, provide opportunities for students, faculty and staff to develop and implement IPM solutions to crop production problems in developing countries. Small grant programs, including the APS Office of International Programs Global Experience, support projects between APS members and developing country partners.

Laboratory methods for detecting and characterizing fungicide resistance

Fungicide resistance testing and monitoring strategies: Good science and common mistakes

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Phytopathology 101:S228

The value of the data obtained when monitoring and testing for fungicide resistance is dependent upon (i) the degree to which the tested samples are reflective of the pathogen population that they purport to represent; and (ii) the extent to which the laboratory assay employed truly reflects the ability of the tested fungicide to inhibit the fungus from infecting and/or developing within its host. With respect to the first criterion, mistakes are most commonly made in terms of interpreting or interpolating the data. Does the detected/reported frequency of resistance pertain to the treated (surviving) or the untreated population? Does the sample represent a local or regional population? Is the sample size adequate to represent the population of interest with reasonable certainty? Mistakes involving the second criterion are most common when in vitro tests either (i) do not measure the activity of a fungicide responsible for controlling disease, e.g., tests for mycelial growth inhibition on a material acting via inhibition of spore germination; or (ii) do not account for processes likely to occur during an infection process in planta, e.g., blockage of an alternative respiration pathway that fungi might otherwise use to overcome the inhibitory activity of Qd fungicides. Furthermore, with respect to fungicides for which quantitative resistance occurs, claims for threshold sensitivity values defined as “resistant” are best supported by relative disease-control data.

Sampling for detecting fungicide resistance

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Phytopathology 101:S228

Fungicides are key components in the control of fungal diseases in many world crops. Laboratory methods to evaluate fungicide sensitivity or resistance have been developed and validated. The sensitivity methods are used to establish baseline sensitivity distribution of a specific fungus to a specific fungicide. The methods are also used in subsequent resistance monitoring to determine possible fungicide resistance development and in checking for cross resistance among fungicides in the same mode of action class. The sensitivity methods must be reliable, validated and must correlate with sensitivity responses in the field. Laboratory fungicide sensitivity
methods are designed according to the biological mode of action of the fungicide that could target e.g. conidia germination or the inhibition of mycelial growth. Fungicide sensitivity assays can be conducted on agar or liquid growth media to check for conidia germination or mycelial growth of non-obligate fungal pathogens. The fungicide sensitivity of non-obligate fungal pathogens can also be tested in vivo on intact plants in a growth room or greenhouse or using detached or leaf disc leaves in the laboratory. In vivo fungicide sensitivity assays are recommended for studies with certain fungicides in certain fungicide classes and in studies to correlate in vivo resistance response to the ones in vitro or field.

Laboratory methods for evaluating resistance for obligate pathogens

Determination of fungicide sensitivity and resistance for obligate fungal plant pathogens presents a difficult challenge. Powdery and downy mildews, and rusts are common targets for fungicide sensitivity testing, and have unique technical problems to address. Spore germination or germ tube elongation tests may be performed for certain fungicides as an in vitro testing method, but in vivo techniques are often necessary. These include the use of fungicide-treated detached leaves or leaf pieces, seedlings or whole plants. Factors to be considered in optimizing these laboratory techniques may include: (i) Proper selection of uniformly susceptible plant tissues, based on age, size or plant variety. (ii) Use of chemicals, such as benzimidazoles, to delay senescence in detached leaves. (iii) The use of a surfactant and appropriate concentrations that allow for uniform fungicide coverage of tissue but do not inhibit the fungus. Furthermore, the obligate nature of these plant pathogens mandates a considerable amount of culture maintenance and sufficient measures must be taken to limit cross contamination between isolates during sensitivity experiments. Unique examples also include deployment of fungicide treated seedlings into production sites and large area monitoring aided by trapping of airborne spores. DNA-based methods are highly useful once resistant isolates have been characterized, and can be utilized to expedite resistance monitoring programs.

Molecular methods for fungicide resistance detection

Determination of fungicide sensitivity and resistance for obligate fungal plant pathogens presents a difficult challenge. Powdery and downy mildews, and rusts are common targets for fungicide sensitivity testing, and have unique technical problems to address. Spore germination or germ tube elongation tests may be performed for certain fungicides as an in vitro testing method, but in vivo techniques are often necessary. These include the use of fungicide-treated detached leaves or leaf pieces, seedlings or whole plants. Factors to be considered in optimizing these laboratory techniques may include: (i) Proper selection of uniformly susceptible plant tissues, based on age, size or plant variety. (ii) Use of chemicals, such as benzimidazoles, to delay senescence in detached leaves. (iii) The use of a surfactant and appropriate concentrations that allow for uniform fungicide coverage of tissue but do not inhibit the fungus. Furthermore, the obligate nature of these plant pathogens mandates a considerable amount of culture maintenance and sufficient measures must be taken to limit cross contamination between isolates during sensitivity experiments. Unique examples also include deployment of fungicide treated seedlings into production sites and large area monitoring aided by trapping of airborne spores. DNA-based methods are highly useful once resistant isolates have been characterized, and can be utilized to expedite resistance monitoring programs.

Molecular methods for fungicide resistance detection

The use of fungicides may lead to the selection of resistant fungal pathogen isolates, despite the precautions taken by the producers and official bodies. Many of the most recent fungicide introductions are single-site inhibitors, which possess a higher risk for resistance development than multi-site fungicides. Fungicide resistance is often based on alterations of the target enzyme, but also other mechanisms could occur (efflux, metabolism or target over-expression). Often point mutations are leading to amino acid exchanges, which are responsible for resistant phenotypes towards QoIs, MBCs, SDHIs, or CAAs fungicides. Recent advances in molecular genetics and its application on an increasing number of fungal pathogen species have facilitated the development of molecular methods for the early detection of fungicide resistance. Different techniques are available, pyrosequencing, Q-PCR, PCR-RLFP, etc. These techniques are especially suitable for detection of single point mutations, but also for cases where this correlation is not perfect and other mechanisms have an influence, such as for the DMI sensitivity shift. The different techniques have advantages and disadvantages, in terms of sensitivity, quantifiability, but also technology input, speed and price. Several molecular methods developed for several pathogens are presented. The consequences for fungicide resistance management and the impact of new technologies on fungicide resistance research are discussed.

**Molecular/Cellular/Plant-Microbe Interactions**

**Biology and Molecular Biology of Closteroviruses**

**Current status of the molecular biology of closteroviruses**

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Phytopathology 101:S229

Closteroviruses make up one of the most economically important groups of viruses of plants, although this was not recognized until recently because of the difficulty of working with these viruses. Their diversity of biological characteristics, which include transmission by different insect vectors, and the fact that some are bipartite with much shorter virions impeded their being recognized as a group. This virus group has members that are vectored in a ‘semi-persistent’ manner by different families of insects: aphids, whiteflies, and mealybugs. Yet each virus-insect interaction is precise. However, recent technolo

**Closteroviruses infecting grapevine in Hawaii**

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Mealybug wilt of pineapple (MWP) is a devastating disease of pineapple, Ananas comosus (L.) Merr., worldwide. The disease is characterized by severe leaf-tip dieback, downward curling of the leaf margins, and loss of leaf turgidity, that can lead to total collapse of the plant. Pineapple mealybug wilt associated viruses-1 (PMWaV-1), PMWaV-2, and PMWaV-3 have been identified in field-grown pineapple throughout Hawaii and are transmitted by the pink and green mealybug mealybugs, Dymicosus brevipes and D. neobrevipes, respectively. In Hawaii, PMWaV-2 infection and simultaneous mealybug feeding are involved in the induction and etiology of MWP, whereas PMWaV-1 and -3 do not appear to be necessary for wilt induction. Genomic analyses reveal that PMWaV-1 and PMWaV-3 lack an intergenic region between the RdRp and the small hydrophobic protein open reading frames (ORFs), lack a conserved motif in ORF4, encode a relatively small coat protein, and lack an apparent diverged coat protein (CPd). These characteristics distinguish them from PMWaV-2 and the ampelovirus type member, Grapevine leafroll associated virus-3 (GLRaV-3). Phylogenetic analyses of seven domains and ORFs from members of the family Closteroviridae show two distinct groups within the recognized genus Ampelovirus. One group of ampeloviruses includes PMWaV-3 and PMWaV-1, and the other group includes PMWaV-2 and GLRaV-3.

**Closteroviruses infecting grapevine**

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Closteroviruses (family Closteroviridae infecting grapevines (Vitis spp.) are a complex and genetically diverse group infecting a single crop species. They are often detected in grapevines affected with grapevine leafroll disease (GLRD). Closteroviruses associated with GLRD are collectively designated as Grapevine leafroll-associated viruses (GLRaVs) and numbered sequentially as GLRaV-1, -2, -3, etc. in the order of their discovery. Among them, GLRaV-3 appears to be the most widespread in many grape-growing regions. Mixed infections of GLRaVs in different combinations are common. The role of these GLRaVs in the biology of GLRD requires investigation. The majority of GLRaVs belong to the genus Ampelovirus, whereas GLRaV-2 belongs to the genus Closterovirus. GLRaV-7 has not yet been assigned to any of the genera in the family Closteroviridae and GLRaV-8 is not a valid virus species. The size of GLRaVs ranges from 13,696 nucleotides (nt) with a relatively simple genome organization encoding seven open reading frames (ORFs) to 18, 498 nt with a complex genome organization encoding thirteen ORFs. A few GLRaVs in the genus Ampelovirus have been shown to be transmitted by different species of mealybugs and scale insect. Recent studies are beginning to reveal new information on molecular biology of GLRaVs in relation to other monopartite members of the family Closteroviridae.

**Novel closteroviruses in small fruit crops**

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Closteroviruses were not known to infect small fruit crops until 2001. Several new members of the family have been characterized in the past decade in

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Role of Fatty Acids and Lipids in Host-Pathogen Interactions

The plant defense hormone jasmonate and its molecular mechanism of action.

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The fatty acid-derived plant hormone jasmonate performs a central role in regulating plant defense responses to biotic stress. Many pathogens and insect herbivores trigger host defense responses by activating the synthesis of jasmonoyl-L-isoleucine (JA-Ile), which promotes the formation of COI1-JAZ coreceptor complexes in which IAZ transcriptional repressors are targeted for ubiquitin-dependent degradation. Mutations that disrupt JA-Ile homeostasis or perception severely compromise host resistance to many insects and pathogens. In contrast to biotic agents that are fended off by the jasmonate pathway, some pathogens, most notably Pseudomonas syringae, exploit the hormone to promote disease. As a potent agonist of the JA-Ile receptor, the P. syringae toxin coronatine triggers jasmonate responses that suppress host immunity. The x-ray crystal structure of the COI1-JAZ receptor in complex with JA-Ile and coronatine has provided an unprecedented view of how this hormone signaling pathway modulates immune function. Recent progress in understanding the molecular mechanism of jasmonate signaling will be presented.

How PI-3-P mediates entry of oomycect, fungal and insect effectors into host cells


Phytopathology 101:S230

Symbionts, both pathogenic and beneficial, must integrate their physiology with that of their host in order to achieve a successful colonization. Effector proteins that enter the cytoplasm of host cells are widely utilized for this purpose by bacterial, fungal, oomycect, protistan, nematode, and insect symbionts. The soybean pathogen Phytophthora sojae, one of the best characterized oomycect pathogens, encodes in its genome nearly 400 potential effector proteins with the cell-entry motif XKLRR. We have recently identified the mechanism by which effector proteins from pathogens and mutualists from two different kingdoms of life, fungi and oomycect, enter the cells of their plant hosts. The mechanism involves the previously undetected presence of the phospholipid phosphatidylinositol-3-phosphate (PI-3-P) on the outer surface of the plasma membrane of plant cells. The virulence proteins utilize PI-3-P as a receptor to gain entry via lipid-raft mediated endocytosis. More recently, we have discovered that two diverse insect pests of plants (hessian flies and aphids) also produce proteins that can bind PI-3-P via RXLR motifs in order to enter plant cells, where they suppress host defenses while the insects feed. We are currently exploring methodologies for disrupting PI-3-P-mediated effector entry in order to create new means for managing oomycect and fungal diseases and insect pests.

Role of glycerolipid metabolism in plant systemic immunity


Phytopathology 101:S230

Systemic acquired resistance (SAR) is a form of immunity that provides protection against secondary infections and involves the generation of a mobile signal at the site of primary infection, which translocates to distal tissues and activates defense responses. We showed that the Arabidopsis acyl carrier protein 4 (ACP4) is required for processing the mobile SAR signal in distal tissues. The acp4 plants generate the mobile signal, but fail to induce systemic immunity in response to it, and this correlates with the impaired leaf cuticle in acp4 plants. Other genetic mutations impairing the cuticle, as well as physical removal of the cuticle in wild-type plants also compromises SAR. Interestingly, a mutation in GLABRA1, a well-known component of trichome development, also affects cuticle development and thereby SAR. More recently, we showed that, glycerol-3-phosphate (G3P), an important and conserved primary metabolite, is a critical inducer of SAR. Genetic mutants defective in G3P biosynthesis cannot induce SAR, but do so in response to exogenous G3P. We show that a G3P derivative is translocated to distal tissues and this requires the lipid transfer protein, DIR1. Conversely, G3P is required for the translocation of DIR1 to distal tissues, which occurs via the symplast. This and the fact that dir1 plants accumulate reduced G3P in their petiole exudates suggest that the cooperative interaction of DIR1 and G3P orchestrates the induction of SAR in plants.

Lipid-mediated cross-talk between plant hosts and fungal pathogens

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Contamination of seed with mycotoxins is a serious food and feed safety hazard as these toxins are potent carcinogens that adversely affect farm animals, poultry and humans. They are produced upon infection of seed by Aspergillus flavus and Fusarium verticilloides. Plant and fungal oxylipins are oxygenated fatty acids produced by diverse oxylipinases including lipoxygenases (LOX). Plant oxylipin function as signals in defense and development. In fungi, oxylipins are potent regulators of mycotoxin biosynthesis and sporogenesis. Recent evidence suggest that plant and fungal oxypins may act as signals in cross-kingdom communication that regulate disease progress. To test this hypothesis, maize and A. flavus oxypin-deficient mutants were created and tested for alterations in the ability of the fungus to colonize seed.
and produce spores and mycotoxins. Additionally, maize LOX knock-out mutants were tested for resistance to other fungal pathogens. Disruption of specific LOX genes resulted in either decreased or increased resistance to leaf blights, stalk rots, and to contamination with mycotoxins with the outcome of interactions dependent on specific LOX mutant and pathogen species. The results will be presented showing that the host and fungal oxylipin metabolism governs the outcome of maize-fungal interactions and that production of plant oxylipins may be modulated by fungi to facilitate pathogenesis and production of spores and mycotoxins.

Chemical ecology of plant-parasite interactions

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What Else Is There? New Genes, Metabolites, and Regulatory Pathways Involved in Biocontrol by Bacteria

Comparative genomic analysis reveals new aspects of the biology and secondary metabolism of bacterial control strains of Pseudomonas spp.

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To explore the genomic diversity of biocontrol strains of Pseudomonas spp., we derived high quality draft sequences of seven strains that suppress plant disease. The strains were isolated from the phyllosphere of pear (P. fluorescens AT506), the rhizosphere of wheat (three strains of P. fluorescens and two strains of P. chlororaphis), or the rhizosphere of peach (P. syxantha BG33R). Genome size varies from ca. 5.9 Megabases with 5500 ORFs (P. fluorescens AT506) to 7.0 Megabases with 6300 ORFs (P. chlororaphis O-6). Along with three previously-sequenced strains of P. fluorescens (P-E5, P-B1 and SBW25), these bacteria share 2831 genes that represent a core genome, with each strain having approximately 300 to 900 genes that are not found in the other genomes. Phylogenetic analysis of the ten strains defined three distinct clades. Within each clade, the strains share 75 to 90% of their proteomes, whereas a smaller proportion of the proteome, typically 60 to 70%, is shared between strains in different clades. Bioinformatic analysis revealed genes with potential roles in the biology of each strain and its multilocus interactions on plant surfaces, including genes for the production of toxins, antibiotics, and siderophores not previously associated with these strains. The many orphan gene clusters in the genomes provide avenues for the future discovery of novel natural products, including those contributing to biocontrol of plant disease.

Novel pathways revealed in P. fluorescens Q2-87 and Q8r1-96


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Pseudomonas fluorescens Q2-87 and Q8r1-96, both from a take-all decline field in Quincy, Washington, U.S.A., are almost indistinguishable in vitro, but only strain Q8r1-96 exhibits the “premier” phenotype distinguished by highly aggressive wheat root colonizing ability essential for the natural disease suppressiveness known as take-all decline (TAD). Despite their phenotypic similarity, comparison of the complete genomic sequences of Q2-87 and Q8r1-96 revealed that Q8r1-96 has 1,373 genes (22.8% of its genome) not present in Q2-87. These include 658 hypothetical and conserved hypothetical ORFs, 88 putative transport and membrane proteins, and various enzymes, toxins, transposases, phages, etc. The two strains probably belong to different serovars; a large O-antigen biosynthesis cluster present in Q8r1-96 is very similar to that from serovar O-5 of P. aeruginosa and likely was acquired by horizontal gene transfer. Both Q2-87 and Q8r1-96 harbor type III secretion systems. The Q8r1-96 genome encodes three effectors, one of which has no known homologues. Q8r1-96 effectors are secreted in culture, injected into plant cells, and can suppress effector- and PAMP-triggered immune responses in tobacco. However, expression of the Q8r1-96 T3SS in the rhizosphere of wheat or pea had only a minor effect on colonization.

What makes Chromobacterium tick? New metabolites from a novel biocontrol agent

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An effective formulation containing the chitinase-producing bacteria including Chromobacterium sp. C61 has been successfully used for control of plant diseases in field. Factors in the culture filtrate showing antifungal activity were extracted into ethyl acetate and fractionation by high-performance liquid chromatography followed by nuclear magnetic resonance mass spectrometry analysis identified the active compound as a novel cyclic lipopeptide. We named this new lipopeptide, containing nine amino acids and the fatty acid, myristate, chromomycin. Transposon mutagenesis of C61 produced mutants lacking antagonism toward the phytopathogen, Rhizoctonia solani. The mutant disrupted gene depD encoded nonribosomal peptide synthesis. The depD mutant had no antifungal activity but produced wild-type levels of chitinase. Unlike other known cyclic lipopeptides, chromomycin and cell-free culture filtrate of Chromobacterium sp. C61 did not possess biosurfactant activity, and swim and swarm motilities of the depD mutant were similar with those of wild-type. In vitro and in vivo bioassay of the mutant of Chromobacterium sp. strain C61 culture and the purified chromomycin showed a broad spectrum of antifungal activity. To our knowledge, this study is the first report to identify a new and novel antifungal compound from biocontrol chitinase producing Chromobacterium species.

Pathogenesis as a mechanism of biological control by Lysobacter enzymogenes

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While there has been a vast increase in descriptions of bacterial pathogenesis of microbial eukaryotes over the past decade, the mechanisms bacteria use to infect these hosts remain poorly defined. The soil bacterium Lysobacter enzymogenes is known as a microbial antagonist and biocontrol agent, functioning presumably through the production of lytic enzymes and secondary metabolites. Recently, however, L. enzymogenes has been found to be a pathogen of fungal hosts, providing a model system to evaluate the role of pathogenesis in microbial antagonism and biocontrol. Recent completion of the L. enzymogenes genome sequence has provided insight into the biology of fungal antagonism. In addition to numerous genes encoding lytic enzymes and antibiotic biosynthesis, genes associated with bacterial pathogenesis, such as those encoding complex effector secretion systems, have been identified. The presence of these latter genes suggests L. enzymogenes employs strategies similar to those used by pathogens of higher plants and animals to infect fungal hosts. Indeed, mutational analysis of selected genes provides evidence for roles of type IV and type VI secretion, as well as type IV pili during infection of fungal cells. Overall, these results support pathogenesis as a mode of L. enzymogenes antagonism toward fungi and provide promise toward developing new and novel approaches for the control of plant diseases.
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Professionalism/Outreach/Industry/Genetic Engineering

Challenges to the Production and Distribution of Quality Planting Materials, Seed, and Seed Systems for Farmers in Developing Countries

Overview of industry’s role in the development of quality seeds

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This presentation will discuss the seed production process used by many commercial seed companies. The process begins with knowing the needs of the customer in each country. Companies utilize an elite set of germplasm, often with a unique set of crop genetics, for producing superior products in that growing environment. These products may be derived from conventional, molecular and/or transgenic breeding programs. They may contain leading edge technology traits for improved resistance to various environmental and biological challenges that a farmer may face during the growing of the crop. All products must meet the regulatory requirements for each country in which they are sold. Utilization of a quality management system ensures that high quality standards and best practices are maintained in the entire seed production process. This includes all stages in the production process: product demand planning, supply planning, field activities, seed conditioning, packaging and distribution. Throughout the growing season, providing technical supportive services to our customers and obtaining their feedback on product performance is an essential part of developing new superior products to help farmers meet the global challenge of feeding the world.

Addressing cereal crops seed supply challenges in sub-Saharan Africa

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Phytopathology 101:S232

A well functioning seed system consists of appropriate variety development, strong production systems, good marketing and sales network and reasonable consumer protection policies. In most of sub-Saharan Africa the system has constraints across each of these areas. To promote the development of seed systems that deliver improved crop varieties to small-scale farmers in a sustainable manner, grants from the Bill and Melinda Gates foundation have provided support to public sector crop breeding programs and small and medium enterprises engaged in seed production and distribution. This has enabled the introduction of high yielding varieties of staple crops that with commercialization would increase the productivity of small scale farmers in select countries in sub-Saharan Africa. In addition, grantees have begun addressing the issue of access and have created awareness with governments and private sector that it is possible to address seed production and availability with the right enabling environment, support to start-up seed enterprises and appropriate monitoring mechanisms.

Overcoming poor seed systems for clonal crops in developing countries

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Productivity of clonally propagated crops is subject to numerous constraints, pests and diseases key amongst them, which readily pass to successive crop cycles through the use of infected planting material. In smallholder systems, where stringent regulations for seed certification are often lacking, infected planting material is regularly used. The development of sustainable seed systems, which addresses the issue of seed health, is critical to overturning yield losses in resource poor smallholder systems. Obvious and/or heavy pest and disease damage is regularly removed from planting material by farmers, however, mildly infected material that is less obvious, is often used inadvertently for planting the successive crop. This perpetuates productivity decline and even loss of germplasm biodiversity through susceptibility to prevalent pests and diseases. In West Africa availability of healthy seed material of yam (Dioscorea spp.) is poor with high proportions of available seed yam infected with nematodes and viruses, and a direct cause of poor yields and erosion of traditional cultivars. Other root and corm based clonal crops are similarly afflicted with pathogens and cryptic pests. To overturn the static or downward trend in productivity of these crops, and loss of biodiversity, innovative IPM options, such as the aeroponically produced potato seed and the bio-enhanced tissue culture technique, developed in a multidisciplinary approach, will become increasingly necessary.

Development of seed technologies and benefits for Africa

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Witchweed (Striga spp.) infestations are the greatest obstacle to sorghum [Sorghum bicolor (L.) Moench] production in many parts of Africa. The development of seed-based technologies to control Striga infestations will provide farmers with new tools to control this parasite. The objective of this project was to develop herbicide seed treatments that could be used to control Striga infestations of sorghum. Our research initially was focused on developing the acetolactate synthase (ALS) herbicide-tolerance trait in the crop. After successfully identifying a native gene that conferred tolerance to ALS herbicides, we evaluated seeds of an ALS herbicide-tolerant sorghum hybrid treated with either imazapyr or metsulfuron-methyl. Seeds treated with the highest herbicide rates had the fewest Striga attachments and the greatest delay in attachment in greenhouse studies. Plants in the untreated control group died at or before flowering due to high levels of Striga infestation. In field trials, herbicide seed treatments, particularly metsulfuron-methyl, delayed and reduced Striga emergence in most environments. Effects of seed treatments on grain yield were variable depending on the timing and severity of Striga infestation. Ongoing crop improvement efforts are focused on introgressing the ALS tolerance trait into Africa-adapted parent lines for use in hybrid development and commercialization.

Seed and seed systems in developing countries and their significance in attaining food security

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Seed is a vital input in a successful agricultural and economic development program. Unfortunately, the value of seed and seed programs is not fully appreciated in most developing countries. Often, seed and grain are not fully differentiated in the eyes of farmers as well as policy makers. Public programs established to administer the production, processing, conditioning, and distributing quality seed to farmers have repeatedly failed in dispensing that responsibility. The value of setting up a private seed sector, driven by the profit motive, and experienced in the science and technology of the
production, processing, and marketing of quality seed and the ability to deliver good quality seed on a timely basis has not been fully recognized. Yet, input delivery systems are indispensable for enhancing impact of investments in agricultural development programs. Science, technology, and innovation can only deliver the expected promise and impact when functional input delivery systems have been put in place. If seed is to serve as a great vehicle of change in transforming a nation’s agricultural development agenda, a public, private, and/or communal seed industry system with capacity for production, processing, certification, and distribution of seed will need to be carefully designed and implemented.

Innovative Chemical and Biological Approaches to Plant Protection
Chemical and gene technological approaches for plant defense activators to control plant diseases
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Plant defense activators (PDAs) are chemicals, which induce a systemic acquired resistance (SAR) of plants against pathogen attack. Although PDAs don’t exhibit direct biocidal activities, they protect plants from a wide range of plant pathogens, have low risk of developing resistance of pathogen and are good in environmental safety. The first PDA of practical use is probenazole (PBZ) followed by acibenzolar-S-methyl (BTH), tiadinil, isolatin and fluoro-thiazoile-ester. PBZ was discovered during in vivo screening for rice blast protectants, and currently has registrations on various diseases for over 10 crops, though a major use is for rice. BTH was found by a special screening technique, which measures the infected leaf area of cucumber plants sprayed with Anthracnose spore suspension. Other 3 PDAs were probably found by more or less utilizing the structural information of BTH. Biological activities of PDAs and their application for control of plant disease as well as advantages and disadvantages of PDAs will be discussed. Extensive studies on the site of action of PDAs in the signal transduction pathway have shown that PBZ stimulates the SAR signaling pathway at upstream of salicylic acid accumulation, while BTH at downstream of it. Recently efforts to discover novel and useful PDAs have been conducted based on the knowledge of defense genes and signal transduction. A high-throughput screening for PDAs and other latest studies will be also introduced.

Strigolactones as chemical signals for plant-plant and plant-microbe interactions in the rhizosphere
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Phytopathology 101:S233

Strigolactones (SLs) are plant secondary metabolites with three unique biological functions. SLs were originally isolated from plant root exudates as germination stimulants for devasitizing root parasitic weeds of the family Orobanchaceae, including PDAs. In Sl-novels, propanes (Orobancha and Phelipanche spp.), and Alectra spp., and so were regarded as detrimental to the producing plants. Their role as indispensable chemical signals for root colonization by symbiotic arbuscular mycorrhizal (AM) fungi was subsequently unveiled, and SLs then became recognized as beneficial plant metabolites. In addition to these functions in the rhizosphere, it has been recently shown that SLs or their metabolites are a novel class of plant hormones regulating plant aboveground architecture. Furthermore, SLs are suggested to have other biological functions in rhizosphere communications and in plant growth and development, in particular, light perception and root system architecture. Since all angiosperms so far examined produce and release SLs into the rhizosphere, any organisms in the vicinity of plant roots may interact with SLs. Their roles are to induce plant resistance mechanisms. In addition to these functions in the rhizosphere, it has been recently shown that SLs or their metabolites are a novel class of plant hormones regulating plant aboveground architecture. Furthermore, SLs are suggested to have other biological functions in rhizosphere communications and in plant growth and development, in particular, light perception and root system architecture. Since all angiosperms so far examined produce and release SLs into the rhizosphere, any organisms in the vicinity of plant roots may interact with SLs. Their roles are to induce plant resistance mechanisms.

Novel technology for termite control based on the dummy-egg carrying behavior
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Phytopathology 101:S233

Social insects rely heavily on pheromone communication to maintain their sociality. Egg protection is one of the most fundamental social behaviours in social insects. The recent discovery of the termite-egg mimicking fungus ‘termite-ball’ and subsequent studies on termite egg protection behaviour have shown that termites can be manipulated by using the termite egg recognition pheromone, which strongly evokes the egg-carrying and -grooming behaviours of workers. Fungal mimicry of termite eggs is one of the most striking evolutionary consequences of insect–fungus association. We found that the termite egg-recognition pheromone consists of β-glucosidase and lysosome. Both enzymes are major salivary compounds in termites and even in a wood-feeding cockroach, and are also produced in termite eggs. The fungus mimics termite eggs chemically by producing the cellulose-digesting enzyme β-glucosidase. Egg mimicry, by which the fungus can easily gain access to the centre of the nest, seems to be an evolutionary loophole around anti-parasite defense in termites. By using the mechanism of egg mimicry, we developed a novel technology to introduce pesticide into the centre of termite nests and destroy the colonies most efficiently.

Microorganisms and plant activators as alternatives to chemical fumigants to control soilborne diseases in Japan
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Worldwide, soilborne diseases are major constraints in production of crops, especially vegetables. Despite societal pressure to reduce chemical fungicide application in the field, few alternatives are available; the need to replace soil fumigants is particularly critical. Biocontrol and biofumigation offer possible microbial-based means to control soilborne plant diseases. Toward that end, several biocontrol agents, such as nonpathogenic strains of Erwinia carotovora, Pseudomonas fluorescens, Varioroxa paradoxus, and Coniothyrium minitans, have been registered as biofungicides in Japan, but their collective market share is small, leaving a substantial unmet need. Another alternative to chemical fumigant is a class of chemicals known as plant activators. These materials do not directly affect pathogens but rather exert their effects by inducing disease resistance in treated plants; therefore they could be effective in controlling soilborne diseases. Validamycin A, which has been registered to control several soilborne diseases, including bacterial wilt of eggplant, appears to be a plant activator, since it is now known to act by inducing plant resistance mechanisms.

Recent development on research and application of novel green pesticides in China: Neonicotinoid insecticides and plant activators as examples
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PEOPLES REP OF CHINA
Phytopathology 101:S233

Some progress have been made in China: 1) Chemo- and bioinformatics for virtual screening; 2) novel targets and new modes of action; 3) environmental and toxic behaviour as well as in vivo high-throughput microscreens; 4) target-based discovery of novel leads with insecticidal, herbicidal, fungicidal, antiviral and eliciting activity. Our group focused on novel neonicotinoid insecticides and plant activators, as serious resistance, high bee toxicity and low lepidoptera activities were found for the former, and rapidly expanding greenhouse agriculture needed new method to control disease for the latter. A binding model of neonicotinoid involving hydrogen-bond induced π-π interaction was proposed and used for design. Very importantly, fixing the nitro group in cis-configuration provided new strategy for design. Introducing fused heterocycle or bulky group are two ways to fixing the direction of nitro. The synthesis, bioactivities in-field and preliminary modes of action of five types of cis-neonicotinoids were investigated in details. Polyfluoro-alkylation of benzothiazoles provided new ways for design of plant cell elicitor and plant SAR activator. The novel leads not only showed high eliciting activities in cell suspension cultures for the secondary metabolites, e.g. taxuymamine, ginsenosides, but also efficiently controlled various plant disease including fungi, virus and soil-borne disease. Their syntheses, bioactivities in-field and preliminary modes of action were investigated in details.

Recent developments in neonicotinoid insecticides for plant protection
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Phytopathology 101:S233

Neonicotinoids (NN) are presently the most important insecticide group, being highly toxic to pests, while low in mammalian toxicity. Some chemicals of this group are hydrophobic to be insecticidal, while NN cover a wide range of hydrophobicity/hydrophilicity and water solibility, by which major use is as plant systemic for piercing-sucking pests, but some are effective as contact
insecticides. Several NN including imidacloprid are highly toxic to the honey bees, while thiacloprid and acetamiprid are low toxic. Such lower bee toxicity is ascribed to high detoxication by the bee. Efforts to include NN in IPM are in progress. NN are analogs of acetylcholine, and for interaction with insect nicotinic acetylcholine receptor (nAChR), but not with mammalian nAChR, several moieties, particularly electron-withdrawing group, are involved without ionization. Such recognition would be the key for further development of NN and possibly neomuscarnoides.

Enhancement of plant growth and plant growth and plant defence activation by Bacillus vallismortis EXTN-1 on various crops

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The efficacy of plant growth-promoting rhizobacteria (PGPR) Bacillus vallismortis EXTN-1 has been proved as biotic elicitor of induced systemic resistance (ISR) against various pathogens in several crops as well as plant growth promotion. The bio-products of EXTN-1 significantly reduced disease severities against bacterial and fungal diseases under greenhouse and field conditions. Various Cyclo dipeptides were identified as elicitors inducing systemic resistance, which were purified from butanol extract of EXTN-1 grown on TSA. Experiments with transgenic tobacco plants carrying pathogenesis-related genes fused with the β-glucuronidase (GUS) reported gene (PR-1α; GUS & PDF 1:2; GUS) showed an enhanced activation of both PR-1α and PDF 1:2 genes upon treatment with EXTN-1. In RT-PCR analysis, both PR1α and PDF1:2 gene expressions were observed in wild type Arabidopsis plants treated with EXTN-1 strain when compared to non treated plants. EXTN-1 with the greatest efficacy under greenhouse condition was tested for the ability to reduce bacterial wilt, fusarium wilt and fruit rot under field condition at various locations in Korea and Vietnam. Besides, EXTN-1 treatment promoted the growth, yield and quality in many field crops better than water treated control plants. Soil drenching or seed priming (106 cfu/ml) of EXTN-1 stimulated seed germination and growth of about 20 crops without any harmful effects which indicate that application of EXTN-1 improves physiological status of crop plants.

International Perspectives on IPM Education for Advancing Sustainable Agricultural Systems

Current status of Integrated Pest Management (IPM) training in universities and other tertiary agricultural training institutions of East Africa

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Phytopathology 101:S234

Integrated Pest Management (IPM) is a relatively new pest management approach, which started taking root in the U.S.A. in the late 1970s. In East Africa however IPM is not widely used as yet. IPM evolved as a result of recognising the dangers associated with extensive and indiscriminate use of pesticides. In attempt to promote this approach, several definitions and concepts were used to describe integrated pest management. Consequently the lack of a simple definition and common understanding of IPM, delayed its being integrated in the curricula of most Universities and institutes of higher learning in East Africa. Similarly integrated pest management has not yet been fully institutionalized at policy level by most of the East African countries. This paper, therefore, reviews the current status of IPM training in Universities in East Africa and explores the extent to which IPM is integrated in undergraduate- and post-graduate degree programs. It also highlights the contribution of the Integrated Pest Management Collaborative Support program of USAID (IPMC/SP) and other international organisations and non-governmental organisations promoting IPM training in Universities and other organisations. The paper addresses the challenges East African Universities have in producing graduates with appropriate IPM knowledge, in a system where extension, research and training functions are handled by different, delinked and poorly funded institutions. Finally it proposes issues that ought to be given further attention to ensure IPM is fully institutionalised in the university education system, national agricultural research system and extension services.

IPM education in India: Training farmers through demonstration

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Phytopathology 101:S234

Practices for preventing pest damage in IPM include inspecting and monitoring crops for damage, using mechanical trapping devices, botanical pesticides, natural predators/parasites, insect growth regulators, mating disruption substances and, if necessary, only need-based and judicious use of chemical pesticides. IPM modules having biopesticides such as Trichoderma, Trichogramma, and neem based pesticides as pest control measures were designed and demonstrated by TERI for paddy, wheat, and sugarcane in more than 25 ha of land in five villages in Shivvalik foothills in Yamuna Nagar district of Haryana. The interventions not only reduced the load of pesticides in the environment but also increased yields and thereby incomes. The success of the project prompted TERI to set up similar demonstrations at other sites and on other crops. Now, IPM modules are being demonstrated for vegetable crops like potato, cabbages, chillies and beans in the state of Uttarakhand on more than 25 hectares of land. Two rears back, initiated a program on okra, eggplant, and tomato in the sates of UP, Karnataka, and AP in partnership with VirginiaTech, U.S.A. under USAID-IPM/CRSP program, have been demonstrated. All necessary support is given to the farmers for procurement and utilization of biopesticides.

Lessons learned in designing IPM education programs for farmers in Central America

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Phytopathology 101:S234

For 25 years Zamorano University outreach programs has been working with small farmers in Integrated Pest Management projects in Honduras, El Salvador and Nicaragua. Our educational programs are tailored to technicians working at public and private extension organization. At the beginning we used the typical short courses with a lot of technical information on pest problems. Giving the social and economic farmers background the first change we did was to present a menu of pest control alternatives for the farmer to choose the ones they can afford and like. Over the last years we move our training program to integrated crop management using farmer field schools for methodological extension methodology. With the participatory extension methodology and the crop focus farmers and organizations are adopting the IPM practices in their crops.

Sustainable intensification of crop production: The essential role of IPM & ecosystem-literacy education for smallholder farmers in Asia

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Phytopathology 101:S234

FAO projects that farmers will have to double food crop production as to meet global food needs by 2050. Asian farmers will be under increased pressure to intensify production and the challenge will be to do so sustainably. Inefficient use of agro-chemicals, both pesticides and fertilizers, remains prevalent among smallholder farmers in Asia. Vital ecosystem services provided by natural biological control are compromised. Promotion of agro-ecology based crop intensification and protection strategies will be essential for global food security. FAO has been working with Asian governments to develop and upscale robust Integrated Pest Management strategies (IPM) for a range of economically important crops during the last two decades. This paper will detail case studies of successfully-employed IPM farmer education strategies, making optimal use of the innovative and adult-education-based Farmers Field Schools. As to achieve global food security, this paper will make the case that it is of vital importance that the millions of Asian farmers become agro-ecology-literate, adopt IPM and become better managers through access to quality education. The paper will also highlight FAO experiences and policy lessons learned, at national and Asia regional level, with regards to the pivotal role of better pesticide regulation and enforcement in tandem with farmer education for sustainable intensification of agricultural production.

Expanding educational and career opportunities for international IPM practitioners

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Phytopathology 101:S234

The global challenge of producing sufficient quantities of food, fiber and fuel safely and sustainably requires implementation of IPM at many educational levels including by those trained at the highest academic level. Doctoral level
MRLs: A Growing Agricultural Export Issue
Pesticide maximum residue limits: Why do they matter?
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Phytopathology 101:S235

Disharmony in pesticide maximum residue limits (MRLs) is hampering international trade for agricultural products. Regardless of whether conventional or organic products, variations in the setting of maximum residue limits around the world is limiting the adoption of new materials for foods that are in international trade. Many countries, including the U.S. and key U.S. trading partners, set their own maximum residue limits for pest control materials using differing risk assessment processes, differing crops covered, and differing priorities. This session will discuss the overall issue of differing international MRLs facing U.S. agriculture including examples of the causes of disharmony, and what efforts are underway to harmonize better. Examples from California almonds, the top U.S. specialty crop in export value, will be included. Additional speakers in this session will provide examples of the impacts of MRL disharmony on their crops and products.

The Pacific Rim maximum residue level (MRL) issues
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Phytopathology 101:S235

Insect and disease pressures often require chemical controls in fresh agricultural production. A primary concern for U.S. growers and shippers when implementing integrated pest management programs, are that, applications of crop protection materials are within pesticide tolerances established in the U.S. but the tolerance may be illegal abroad. As this trend continues to grow, fresh market commodities face challenges in managing insect and disease control to meet export phytosanitary requirements while observing the differing regulatory requirements for residues within foreign market destinations. Current situation indicates that U.S. MRLs are frequently higher than established foreign MRLs as a result of the differing methodologies used. U.S. commodities have significantly more MRLs established compared to tolerances available within foreign markets. U.S. growers consider MRL harmonization one of the most important and growing issues within international agricultural trade. Continuing and new MRL challenges for growers and shippers of fresh agricultural commodities include key market destinations for U.S. production. There is movement in the Pacific Basin toward establishing national MRL lists; some transitions are orderly, others are not. New insects and disease continue to increase the pressures on the MRL issues, along with private sector concerns and standards which are becoming increasingly common within the U.S. agricultural landscape.

MRLs in Europe—How philosophies differ from the United States
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Phytopathology 101:S235

What are Maximum Residue Levels (MRL)? Why would an American Phytopathological Society be interested in MRLs? This presentation will go over a brief background on MRLs and then discuss the impact on trade with the EU resulting from unharmonized MRLs. The U.S. process for establishing MRLs will be compared and contrasted with the European Union process. The recent EFSA Reasoned Opinion announcing the reliance on Codex MRLs will be examined. The impact of Codex MRLs on the U.S. grower’s ability to trade with the EU will be presented. Specific U.S. MRL issues will be addressed and opportunities for harmonization will be highlighted.

MRL Challenges: Tree fruit exports from the Pacific Northwest
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Phytopathology 101:S235

The Pacific Northwest states of Idaho, Oregon and Washington export 1.45 billion pounds of apples and 298.5 million pounds of pears to over 60 countries and 80 million pounds of cherries to 48 countries. This volume represents approximately 30 percent of the annual production of each of these crops. Northwest Horticultural Council works to ensure minimal trade disruption by monitoring foreign government regulations with regards to maximum residue levels (MRL) on these fruits and provides prepared information to the Pacific Northwest deciduous tree fruit industry prior to export season. Pest control consultants, growers and exporters review this data to track certain pesticides which can be used to reach the maximum number of export markets. Top export markets are also closely monitored for changes in their MRL systems and reported as “country alerts” through a website that can be accessed by industry growers and shippers and the public at large, including foreign countries. Each month there may be individuals from over 100 countries visiting the site. Working with U.S. trade representatives, regulators, chemical manufacturers and registrants and agencies of foreign governments, our industry has been successful in gaining some critical MRL values and closing gaping holes in positive MRL lists that limit the export of our produce. Specific examples of how our industry approached gaining MRLs and discussion on the limitations still present in the system will be presented.

Pesticide Resistance in Agriculture—A Global Issue
IRAC global industry leadership to preserve insecticidal chemistries through education, maintaining insect susceptibility, and managing insect resistance
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Phytopathology 101:S235

Effective management of insect pest populations in most of the world’s agriculture, horticulture, and human safety is dependent on a variety of inputs including a ready supply of safe, highly efficacious chemical insecticides. With their abundant numbers and short life-cycles, populations of pest insects can readily develop resistance to the insecticides used against them with the result that once effective insecticides are no longer able to control the pests for which they were intended. The Insecticide Resistance Action Committee (IRAC) was formed in 1984 to provide a coordinated crop protection industry response to prevent or delay the development of resistance in insect and mite pests. The mission of IRAC is to facilitate communication and education on insecticide resistance and to promote the development of resistance management strategies in crop protection and vector control so as to maintain efficacy and support sustainable agriculture and improved public health. IRAC is an inter-company organization comprised of over 15 chemical companies acting as a Specialist Technical Group of CropLife International. IRAC International supports resistance management project teams but also provides a central coordination role to regional, country, and technical groups around the world. IRAC International’s main focus is on education, communication and regulation of insecticide resistance management. Various examples of IRAC globally supported activities will be presented.

Fungicide RAC approach to resistance management
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Phytopathology 101:S235

The Fungicide Resistance Action Committee (FRAC) is an industry group reporting to Crop Life International. Its purpose is to identify potential and
existing resistance problems, evaluate scientific data and knowledge, and provide fungicide resistance management guidelines and education to prolong the effectiveness of fungicides and limit crop losses should resistance occur. Fungicide resistance management strategies are based around good agronomic practice, optimum fungicide timing, the use of appropriate dose rates, and optimizing the use of alternative modes of action in fungicide programs. Since target site mutation is the predominant mechanism of resistance to fungicides, using alternations and mixtures of fungicides having different modes of action is common practice in successful disease and resistance management strategies. This approach depends on the availability of diverse modes of action, a challenge in today’s regulatory environment. Increased regulatory hurdles make the discovery and development of novel chemistries more difficult and costly, as well as potentially removing existing modes of action, (including the multisites) from the market. A further issue is the continuing trend in the food chain to demand fewer active ingredients in crops, although these are at residue levels within the accepted MRLs. The use of mixtures and alternations of fungicides is the foundation of most resistance management programmes; maintaining this approach will be vital for the future.

Herbicide RAC view of resistance

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Phytopathology 101:S236

The Global Herbicide Resistance Action Committee (HRAC) is dedicated to developing and promoting best practices to manage herbicide resistance. Collaborations across the industry and with the academic community are central to promoting the adoption of best management practices by farmers. Herbicide resistance is common to most classes of herbicide chemistries and most weed species. However, there are differences in number of resistant species and probability of resistance between the classes and/or individual herbicides. In spite of resistance in many herbicide classes, products with known resistance remain important to agriculture. Stewarding the use of each herbicide through proactively managing resistance across all herbicides is important to agricultural productivity and sustainability. Over reliance on a single herbicide across multiple seasons in the absence of other herbicides and weed management options is not a sustainable practice. Sustainability of all products can be achieved through the implementation of diversified weed management programs. There are multiple management options that can be tailored to fit different farming situations while addressing environmental and socioeconomic needs. The identification and promotion of diversified programs is a focus of HRAC and public sector weed scientists worldwide. The current status of herbicide resistance worldwide will be reviewed, along with programs and options for implementing diversified programs.

Gene flow and herbicide resistance: Lessons learned from herbicide-resistant rice systems

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Phytopathology 101:S236

Gene flow in plants is a process whereby genes are exchanged between members of the same or closely related species via pollen and become established in new populations. This natural process has long been an issue in breeding and the seed industry, but interest has increased since the deployment of pesticide-resistant crop systems in areas infested by weedy/wild relatives of the crop. Imidazolinone (IMI) herbicide-resistant (HR) genes imbedded in traditional cultivars or in commercial hybrids of rice are examples of such systems, which are now widely grown and have been highly successful in the U.S. The types of rice and weedy rice can affect outcrossing rates in this self-pollinated species. Outcrossing has ranged from <0.001% to >1.0% as shown using SSR analyses, and occurs primarily between plants separated by < 2 m. Outcrossing was detected at 300 m in a field-scale experiment. Some bottlenecks that restrict gene flow between rice and weedy rice have been identified. However, repeated applications of IMI herbicides to rice fields over time result in powerful selective advantages to weedy rice plants once they have acquired HR genes. Planting time, humidity and temperature during flowering, and other environmental factors also affect outcrossing and gene flow. Reports of HR weedy rice in farm fields are increasing. The degree to which HR genes have introgressed into weedy rice populations via gene flow from IMI rice, and approaches to mitigating this process are under investigation.

Herbicide resistance as a threat to dryland farming in the Mediterranean

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Phytopathology 101:S236

Chemical weed control in non-irrigated (dryland) arable crops is necessary due to the high infestation with winter broadleaf and grass weeds. The harsh environmental conditions and lack of suitable alternative crops impose the necessity to grow mostly wheat or barley as monoculture interrupted with fallow years. Farmers reduce soil tillage and rely more on chemical weed control. Herbicides are often used repeatedly as a single mode of action in arable crops for many years imposing high selection pressure on the weed populations. These misuse of herbicides resulted in the inevitable evolution of weeds resistant to ALS, ACCase and EPSPS inhibiting herbicides. Several population evolved resistance to more than one mode of action (multiple resistance). Altered target site and metabolic ALS resistance was detected in both broadleaf and grass weeds. ACCase resistant weeds such as Lolium rigidum, Phalaris minor, P. paradoxa and Avena sterilis are widely spread in the Mediterranean. Resistance to glyphosate was confirmed in L. rigidum and Conyza bonariensis. The cost of chemical weed control often drives the farmers to apply herbicides below the recommended use rate, facilitating the evolution of non-target site resistance. The fast evolution and spread of herbicide resistant weeds endangers the sustainability of arable crops in various Mediterranean countries emphasizing the crucial need for integrated weed management practices.

Managing glyphosate resistant weeds in dicamba resistant soybeans

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Phytopathology 101:S236

Glyphosate is a highly efficacious and economical broad spectrum herbicide widely used in agriculture. The heavy reliance of glyphosate for weed control has led to an increase in the number of resistant weeds world wide. Recommendations for managing glyphosate resistant weeds include the use of herbicides with alternative modes of action, pre-emergence application of residual herbicides, as well as incorporating agronomic practices such as tillage. Dicamba is a member of the synthethic auxin family and is efficacious on dicotyledonous weeds. Dicamba is effectively used in corn, which is naturally tolerant; however, soybeans are extremely sensitive. Engineering crop safety to dicamba permits its use in-crop for effective control of dicotyledonous weeds and expands weed control options for the growers. In this presentation, I will first summarize the current understandings on glyphosate resistant mechanisms, followed by our efforts to engineer dicamba resistance in soybeans through a deactivation mechanism. Soybean plants transformed with a microbial mono-oxygenase gene withstood high rates of dicamba (> 0.55 kg/ha) showing no visible injury in multi-year, multi-location field trials. We will also present greenhouse and field data showing how dicamba can be used in conjunction with current weed control systems to control glyphosate-resistant dicotyledonous weeds.

The current state of resistance to acetohydroxyacid/acetolactate synthase inhibitors

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Phytopathology 101:S236

The acetohydroxyacid/acetolactate synthase (ALS) inhibiting herbicides are used for weed management in multiple crop and non-crop situations. ALS inhibiting herbicides were introduced in the early 1980s and quickly came to dominate many cropping situations. However, the use of ALS inhibitors selected for herbicide resistant weed populations within a few years after growers began to apply these herbicides over broad areas. Although ALS inhibitors are extremely effective herbicides, multiple mutations can occur at the target site, acetohydroxyacid synthase, that prevent the binding of the inhibitors. Currently ALS inhibitor resistance has been selected in 107 species and in over 300 biotypes. There is more resistance to ALS inhibitors than there is to any other mechanism of action. However, ALS inhibitors are still widely used and still provide effective weed management. New herbicides that are ALS inhibitors continue to be registered, in spite of the fact that resistance can be quickly selected. The introduction of glyphosate-resistant crops slowed the selection of ALS inhibitor resistance in major crops. The selection of glyphosate resistant weed populations has resulted in the re-introduction of ALS inhibitors into weed management programs. The judicious use of ALS inhibitors in an integrated weed management program can retain the effectiveness of these herbicides while still managing resistance.
Using Translational Biotechnology to Deploy Disease Resistance Traits in Crop Plants

Risk assessment studies: Insights into the safety of disease-resistant transgenic horticultural crops
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Phytopathology 101:S237

Disease-resistant horticultural crops were amongst the first genetically modified crops to be deregulated in the United States in the mid 1990’s. To date, virus-resistant vegetable (summer squash) and fruit (papaya and plum) crops are commercially available. Safety issues have been expressed during their development and release, although most of the risks are the same or similar to those posed by traditionally bred plants with host resistance. Considerable risk assessment research with virus-resistant horticultural crops has shown little to no detrimental impact on the environment and human health beyond those of traditionally bred counterparts in natural and traditional agriculture settings. There is also a documented safe release of virus-resistant transgenic summer squash, papaya and plum over the past 13–15 years. Our current knowledge of risk assessment infers that safety issues should not hinder the release of new virus-resistant transgenic horticultural crops. Transgenic summer squash and papaya have become important components of disease management programs and the prospects of further advancing transgenic vegetable and fruit crops for practical disease control are very promising.

Transgenic squash: The inside story
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Phytopathology 101:S237

Transgenic squash has been available commercially since 1995. Since that time, it has provided an effective means of virus control for squash growers. This presentation will review the history of development of this product, the regulatory issues that were raised that are still relevant today, and the research conducted since commercial release that address those issues. The status of the development of transgenic specialty crops will also be discussed.

An ethical look at integrating new traits using biotechnology—A nonscientist perspective
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Phytopathology 101:S237

Much is at stake in the development of plant biotechnology. Genetic engineering crucially affects a host of vital issues including food security, consumer rights, environmental protection, human health, trade laws and our relationship to the natural world. Because of its vast significance, the genetic modification of plants has been widely debated both in the United States and internationally. Both the advocates and opponents of GM food appear equally intransigent, with the prospect of reconciling the two sides seemingly bleak. What has often been missed in the biotechnology debate is adequate discussion of the moral and cultural issues associated with conducting genetic engineering responsibly. Biotechnology is not an isolated scientific process but a social practice that involves different contexts, actors, relationships and dynamics of power. The complex web of relationships that make up biotechnology require that the ethical obligations human beings have to one another, the environment, and future generations, both human and nonhuman, be central in discussions about genetic engineering.

A resistance gene from pepper confers effective field resistance to bacterial leaf spot in tomatoes
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Phytopathology 101:S237

Resistance to bacterial leaf spot disease (BLS), caused by Xanthomonas spp., is conferred by several genes in pepper. One of these, known as Bsr2, confers recognition to the highly conserved effector, AvrBs2. We investigated if the Bsr2 gene could confer resistance to its close relative, tomato, which is severely afflicted by BLS throughout commercial growing areas in Florida. We transformed tomato plants with Bsr2 and inoculated transgenic lines with the predominant tomato BLS race, X. perforans race T4, to assess resistance in greenhouse and field trials. Field trials were conducted over five years and two environments comparing transgenic lines, the non-transformed parent line, several widely used Florida commercial varieties, and a number of wild tomato relatives with BLS resistance. In every trial, Bsr2 transgenic lines were found to have significantly less disease than the other varieties tested. Assessment of yield revealed that Bsr2-containing lines had significantly greater fruit production than the susceptible parent line. These data demonstrate that inter-generic transfer of this resistance gene between two comestible crops is successful and provides effective, environmentally-benign protection to tomatoes in the field. It is anticipated that deploying this genetic resistance commercially will avoid significant yield losses caused by BLS and provide an opportunity to eliminate and suspend use of ineffective and environmentally harmful crop protection compounds.

History of the successful introduction of transgenic virus-resistant papaya in Hawaii
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Phytopathology 101:S237

In May 1992, papaya ringspot virus was observed in Puna District on Hawaii Island where 95% of the state’s papaya was being grown. The virus rapidly spread through Puna and by 1995 essentially all of the papaya orchards in Puna were infected. Since 1992, Hawaii papaya production had decreased by 50% by 1997. In May 1998, eight years after the discovery of the virus in Puna, the virus resistant transgenic Rainbow and SunUp papaya were released, and essentially saved the papaya industry in Puna. However, the transgenic papaya story in Hawaii had already started back in 1985 when efforts were begun to develop a virus resistant papaya via the concept of pathogen derived resistance. By 1991, greenhouse studies showed that a transgenic line was resistant, an initial field trial was instituted on Oahu Island in 1992, another one in Puna in 1995 where the virus had eliminated production on a farm, and efforts to deregulate the transgenic papaya were started in early 1996. Licenses to commercialize the crop were obtained and seeds were released free to farmers on May 1, 1998. Thus started the transgenic papaya era in Hawaii. Today, roughly 85% of Hawaii’s papaya is transgenic. The Hawaiian transgenic papaya story represents a collaborative effort between institutions and a small group of scientists that were committed to help save the Hawaiian papaya industry. The ‘inside’ story of this effort will be presented.

Weed Science

Invasive Weeds as a Threat to Agriculture and Human Health
Ambrosia spp.: Weed management and human allergy
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Phytopathology 101:S237

The main cause of allergy and pollen asthma in North America and Central Europe is pollen from ragweed (Ambrosia) a genus in the Asteraceae. Currently short or common ragweed (Ambrosia artemisiifolia, L.) is rapidly spreading in Europe and has the highest weed densities in the Carpathian basin: Croatia, Hungary, and Serbia. Despite continuous efforts by the Hungarian government during the last ten years to eradicate ragweed, levels of its pollen in the air did not diminish. Ragweed infestation is heaviest in sunflower (Helianthus annuus L., the third most important crop in Hungary) fields, producing the overwhelming majority of allergic pollen in the air (in the end of the summer pollen counts reach 1000 grains m⁻³) even in urban areas. In the presentation we show the current situation in Hungary, the most recent measures, and the strategic program based on the remote sensing and precision weed management we developed for controlling ragweed and suppressing its pollen production.

The need for weed risk assessment
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Phytopathology 101:S237

Invasive plant species affect health and agriculture, their impacts are second only to habitat destruction in terms of loss of biodiversity. The most cost-effective means to manage exotic weeds is prevention. The basic instruments for prevention are phytosanitary legislative framework, weed risk assessment
Towards the sustainable management of parthenium weed (Parthenium hysterophorus L.) under a changing climate: An international collaborative approach


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Phytopathology 101:S238

Parthenium weed (Parthenium hysterophorus L.) is a weed of global significance and has become a major weed in Australia, India, South Africa, Ethiopia and Pakistan as well as many other countries of the world. It has serious impacts upon human and animal health. No single method alone has proven effective in its management. Through international collaboration aspects of the biology and ecology of this weed have been investigated to help develop sustainable management strategies that can be used now and in the future under a changing climate. Initial work has assessed the reproductive potential of the weed, its seed bank size and persistence. The mode of spread both nationally and internationally, and how it affects plant community biodiversity, has been examined. New management strategies are based around preventing seed spread and reducing population size in heavily infested areas. These latter approaches are built around biological control using both introduced natural enemies (insects, pathogens), which contribute significantly to the reduction of the weed, and suppressive plants, and studying their interaction. Mapping approaches are being used to monitor spread, identify future locations that may be at threat, as well as determining effectiveness of the management approaches. On-going work is being conducted on the genetic make-up of parthenium weed populations around the world, their likely susceptibility to biological control, and their mode of spread.

Invasive weeds—A global overview

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Phytopathology 101:S238

Invasive plants can be problematic in both agricultural and non-agricultural environments. Because transportation corridors provide rapid and easily accessible routes of introduction to many areas around the world, invasive plants are common to most countries. While the focus of invasive species in developing countries is on agricultural pests, more developed countries or islands give equal, if not greater, emphasis on invasive plants of non-crop ecosystems. There are many examples of the impacts of invasive plants throughout the world. *Herculeum mantegazzianum* causes severe human phytophotodermatitis. Aquatic plants can increase the risk of West Nile virus risks, interfere with boating activities, or block the movement of water to crops in irrigation systems. The climbing perennial vine *Mikania micrantha* has spread rapidly in Asia and seriously threatens tree crops. Parasitic weeds can dramatically reduce crop yields, particularly in Africa. *Lantana camara* has impacted forest regeneration. Rangelands in the U.S. has been invaded by *Bromus tectorum*, which alters fire cycles and reduces forage capacity. On tropical islands *Miconia calvescens* greatly reduces native plant diversity. The impact of invasive plants is significant worldwide and can compromise both human interests, as well as natural ecosystems. As a result, many countries are becoming more active in dealing with the threat of invasive plants by implementing programs to manage invasions and prevent future introductions.

Invasive weeds in the Mediterranean region

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Phytopathology 101:S238

The Mediterranean region is facing global climate changes as well as other parts of the universe. According to NASA information, this region is very likely to have less precipitations, more drought and more hot days and nights. These growth conditions are favorable for the invasive species found in the region during the last three decades, especially along with a massive import of grains for human consumption or animal feeding. Among the invasive species spread in the region are: *Acacia spp.*, *Solanum eleagnifolium* and members of the COMPOSITAE; *Ambrosia spp.*, *Verbesina encelioides*, and *Heterotheca subaxillaris*. *Ambrosia* spp. is considered as the major cause of human allergic due to its male flower pollen. Perennial species of this genus e.g.; *A. confertiflora* and *A. tenuifolia* threatens field crops and orchards produce underground rhizomes and much more seed per plant. *V. encelioides* is spreading in this region regardless of the fact that seeds can survive drought and high temperatures and high germination rates observed with seeds at the soil surface. *V. encelioides* is a drought tolerant, require exposure to light to establish, prefers sandy soils and tolerates alkaline soils. Considering the invasiveness of *V. encelioides* in Morocco and Israel and its resistance to drought, the Mediterranean region is considered to be at risk. This plant is reported as a weed in many crops (maize, peanut, orchards, vegetables, etc.) which are cultivated in some parts of the southern area.

Ability of native insects in Hungary to suppress the spread of common ragweed (*Ambrosia artemisiifolia* L.)

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Phytopathology 101:S238

Common ragweed (*Ambrosia artemisiifolia* L.) was first detected in Hungary in the early 1920s. Under favourable climatic and environmental conditions during the past 60 years it became the most frequent weed species, covering 5.3% of the arable crop area. Now it is present on 5 million of the 6.5 million hectares of arable crop area of Hungary. Surveys were conducted for indigenous insects associated with common ragweed in Hungary. The most frequently occurring insects were *Cyclaids* (25%), followed by plant bugs (*Heteroptera* (22%). *Proportions of Iles (Dyptera), Hymenoptera, and spiders (Araneae) were equally ca. 9 percent. Beetles (*Coleoptera*) made up 8 percent of the total catch, followed by thrips (*Thysanoptera*) 5%, psyllids (*Psyllidae*) 4%, butterflies (*Lepidoptera*) and aphids (*Aphididae*) 2-2.5%, and *Collembola made up 4% and others 1%. The majority of the collected species were polyphagous. Apart from plant bugs, psyllids and aphids most species were univoltine, whereby their feeding damage was not sufficient to fulfill the requirements for biological control agent candidates. In addition, three aphid species were found feeding on common ragweed. Among these, *Brachyciauda helichrysi* (Kaltenbach) caused chlorotic spots and leaf distortion on infested plants. On rare occasions, *Aphis fabae* (Scopoli) formed dense colonies on ragweed stems and *Myzus persicae* (Sulzeter) was found on the lower side of the fully developed leaves of the without causing any visible symptoms.

Parasitic Weeds—The Drawback of the Hungry World

Striga—A formidable challenge to Africa’s food security

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Phytopathology 101:S238

The parasitic witchweeds (Striga spp.) cause catastrophic crop losses in cereal and legumes, the staple crops in Africa. Vast areas of the crop lands of Africa are highly infested with Striga with diminishing productivity or ruled unfit for cultivation and abandoned. The problem has been most severe in the semi-arid tropics, where the poorest of the poor reside. Control of Striga through conventional weeding has not been effective as the root-parasite causes great damage before it emerges above ground. Decades of research has generated a number of control and management approaches via good crop husbandry practices as well as chemical, genetic, and biological options. Where appropriately deployed, these control measures have produced effective relief and improved crop yields, though not resulting in eradication of the parasite particularly after severe infestation. Eradication will require sustained use of a mix of approaches over a long period of time, and strategically combining methods that offer immediate relief and increased crop yield with those that target the decrease of the Striga seed reserve in the soil in an integrated Striga management (ISM) approach. Experience in the development and use of such approaches will be shared.
Broomrape management–difficulties and solutions
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Phytopathology 101:S239

The Orobanchaceae consists of 15 genera of chlorophyll-lacking root holoparasites including the genus Orobanche and Phelipanche (common name broomrape), that include more than 100 species that parasitize broad-leaf plants, extracting from them nutrients, minerals and water. Broomrape species infest wide ranges of crops around the globe, mainly in Mediterranean and Mediterranean-like climates causing severe crop yield and quality losses and pose a major threat to the food security of numerous communities. Broomrape is difficult to control due to its subsurface location, intimate association with host roots, lack of chlorophyll and complex mechanisms of seed dispersal, germination and longevity. Control measures include soil fumigation, soil solarization, catch and false host crops, hand weeding, selective herbicide application, crop seed coating, resistant cultivars, biological control agents and herbicide resistant crops. Even though major advances have been made in broomrape research, in most cases this knowledge has not developed to date into efficient cost-effective control measures that have percolated to farmers, especially in developing countries. An integrated large scale regional and national control approach utilizing seed bank eradication, the employment of existing and new genomic and biotech based control tools, together with phytosanitary and regulation measures is essential to combat broomrape parasitism and insure long term sustainable control.

Selective and non-selective management of field dodder (Cuscuta campestris) B. RUBIN (1)
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Phytopathology 101:S239

Field dodder (Cuscuta campestris Yuncker) is a nonspecific above-ground holoparasite, totally dependent on a host plant for assimilates, nutrients and water surplus. The parasite is widely distributed, causing yield losses to a large range of host plants. Seed remain viable in soil for many years due to a thick and impermeable seedcoat. The parasite is extremely difficult to manage due to the association and connection via the vascular system between the host and parasite. Prevention of dodder seed distribution with crop seeds or machinery and removal of neighboring weeds are the most effective and most economical methods for Cuscuta management. The host-parasite connection and proximity make selective chemical control very difficult. Due to the low transpiration rate of the parasite, herbicides that are xylem-translocated in the host’s may not reach the parasite in sufficient amount to kill it. On the other hand, phloem mobile herbicides applied to the host may accumulate selectively in the parasite that acts as “super-sink”, may inhibit the parasite growth without harming the host. The most effective measures for dodder control are herbicides that inhibit the early growth and development of the parasite (e.g., germination and emergence) such as inhibitors of cell division. Unfortunately, biological control using plant pathogens or insects and the use of transgenic herbicide-resistant crops is limited and less efficient. The need for effective dodder management practices is crucial.

Role of strigolactones in the host-parasite association K. YONEYAMA (1), X. Xie (1), K. Yoneyama (1)
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Phytopathology 101:S239

Witchweeds (Striga) and broomrapes (Orobanche and Phelipanche) are the two most devastating root parasitic weeds belonging to the family Orobanchaceae. These root parasites have evolved unique strategies to ensure their survival and their life cycles are closely associated with those of their host plants. The initial step of such host-parasite interactions can be seen in the seed germination. The seeds, however, will not germinate, unless they are exposed to chemical stimuli by germination stimulants produced by and released from plant roots. The majority of the stimulants that have so far been identified are strigolactones (SLs). Since plants produce and release trace amounts of SLs that decompose rapidly in the soil, only the parasite seeds in the host rhizosphere can perceive SLs and thus germinate. So far, nearly 20 SLs have been characterized and more than 100 SLs may exist in the plant kingdom. In addition, plants produce and release mixtures of SLs into the rhizosphere, suggesting that the profile of a SL mixture may be unique to the producing plant and its biological status. Indeed, growth conditions and environmental factors such as light, temperature, humidity, and, in particular, nutrient availability, have profound effects on SL production in plants. Accordingly, it is likely that root parasites recognize their hosts by sensing qualitative and quantitative differences in SL mixtures released from plant roots.

Plant Protection and Food Security in a Changing World
New challenges for plant protection under conditions of climate change J. H. MCBEATH (1)
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Phytopathology 101:S239

Plant protection and food security are strongly influenced by climate. Changes in atmospheric and soil temperatures, precipitation, air currents, and storm surges have physical, chemical and biological implications for the macro- and micro-flora and fauna of ecological and agricultural systems. These impacts affect conservation of species; their composition and abundance, the relationships among prey and pathogens/predators and trophic levels. Some invasive alterfire regimes, causing additional damage to ecosystems. Changes adversely impact crop adaptation and predispose them to damages caused by pathogens and insect pests. In arctic and sub-arctic regions, climatic change benefits crop production as environmental conditions become milder and more hospitable. In a new climate scenario, invasive pathogens, insects and weeds may become established and affects productivity. Early detection and rapid response are tools for their eradication and mitigation. All of these are likely to have impacts on the collective food production of a nation, its capability to feed its people and degree of dependency on other nations for food supplies. As global trade expands, there also are increased costs and challenges to protect countries from threats of invasive species. It would be to the interest of each nation to thoroughly understand the flux of pathogens, insect pests and weeds under changing climatic conditions. Sharing this information openly will reduce the risk of new invasive threats internationally.

Snow molds in a changing environment and molecular basis for their interactions with plants under the snow A. TRONSMO (1), R. Imai (2)
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Phytopathology 101:S239

Plant production in sub arctic area is assumed to be greatly affected by climate change. A potential for increased biomass production is expected due to higher temperatures in the growth season. However, diseases previous restricted by a hostile climate will develop, and existing diseases may appear in a new “costume”. Snow molds are by some foreboded to disappear. However, this prognosis does not take in account that resistance to snow mold fungi to a large extent depends on cold hardening of plants, a condition that resembles acquired disease resistance. In northern climate, plants respond to

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decreasing temperatures in fall by metabolic changes that enhance their resistance to most winter stress factors, including resistance to snow moulds as well as other fungal diseases. This process includes accumulation of defense related proteins. Disturbances of the natural cold hardening will render current varieties of plants more susceptible to diseases that are favored by the changing climate. In order to understand how cold induced resistance in plants affects the fungal infection process, a model system based on ecotypes of Arabidopsis thaliana and the snow mold Typhula ishikariensis has been developed. Using this system, it was shown that cold hardening enhances resistance against snow mold in Arabidopsis. Arabidopsis plants over expressing a cold-inducible cystatin from wheat, TaMDCI, were more resistant against T. ishikariensis than wild type plants under conditions mimicking snow cover.

Climate change and plant protection: Emerging viral and weed threats
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Phytopathology 101:S240

The increase in the introduction of new, invasive pests (pathogens, fungi, weeds and insects) represents a significant challenge to USDA in maintaining a secure, safe and adequate food supply. Although invasive biology has become the focus of a number of research efforts, no systematic evaluation of how climate change and/or rising atmospheric carbon dioxide can alter their establishment, success and impact on food security is available. Yet we recognize that human-induced climatic change associated with weather extremes, precipitation, temperature and carbon dioxide is almost certain to extend the range and increase the impact of agricultural invasive species. There is an urgent need therefore to assess the vulnerability of agriculture to climate-induced changes in invasive species biology. Vulnerability can be defined as the measure of the potential impacts of a given change, minus the adaptive capacity to respond to that change within the system being affected. In this overview I will provide illustrative examples regarding how global climate change and rising carbon dioxide has and will alter the vulnerability of agriculture to invasive species. I will also emphasize that the information needed to fully assess the vulnerability of the U.S. food supply to such threats is lacking. I provide a series of recommendations that I hope will begin to address these vulnerabilities and to maintain food security in an uncertain climate. This information should be of interest to a wide range of stakeholders, including land managers, policy makers, educators and scientists.

Climate change: Impact of invasive arthropods and pathogens on food security
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Phytopathology 101:S240

Pests in all taxa cause more than $140 billion in losses annually in the U.S.A. (Pimentel et al. 2000), and billions more worldwide. These losses may be exacerbated by climate change. However, predicting the geographic distribution and dynamics of pests and their impact in time and space is difficult, and the complexity may increase with global change. Local dynamics of crop plants, pest arthropods, pathogens and weeds are determined by weather and interacting species, with climate determining the geographic range. Methods used to predict the range fall under the ambit of ecological niche models (ENM) that may be statistical, physiological or use artificial intelligence concepts. ENMs characterize the ecological niche of a species to estimate its potential invasiveness and range in native and in new areas, or under climate change scenarios. Deficiencies of ENMs may be resolved using weather-driven physiologically-based demographic models (PBDMs) that include the bottom-up effects of plant dynamics, and the top-down action of herbivores/pathogens and natural enemies. PBDMs capture the biology of the interacting species and use observed weather or climate model scenarios to drive the model dynamics across space and time. The same concepts and models may be applied at all trophic levels, including the economic one. PBDMs can be used as the economic objective function for bio economic analyses at local or larger scales under current climate and climate change scenarios.

Benefits and pitfalls of changing host environment for the purpose of plant protection
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Phytopathology 101:S240

Agriculture is a 'system' where there are favorable and unfavorable factors in each of the four 'environments' (plant, physical, biological, and pathogen) that determine the severity of plant disease. Any change within any one of these environments can drastically change the outcome for disease because of the dynamic nature of the interactions. Specific changes are basic to our understanding and cultural control of plant disease. A few degrees difference in the temperature of irrigation water can mean the difference between severe Pythium root rot and little disease of cotton. A small change in temperature around 26°C can render a small grain plant physiologically susceptible to Fusarium head blight (FHB) by changing its C and N metabolism. Very low concentrations of the glyphosate herbicide [N-(phosphonomethyl)glycine] can cause physiological changes similar to temperature to predispose plants to FHB and other diseases while providing a transient resistance to obligate rust pathogens. Surfactants can also change the host environment to facilitate penetration of Clavibacter, Xanthomonas and other pathogens. Nutrient management is a powerful way to alter the host environment so it is unfavorable for pathogenesis. Understanding the mechanisms of environmental change within the host are important so that predisposition in one area can be countered by changes in another to maintain disease suppression for efficient and effective plant protections.
2010 North Central Division Meeting Abstracts

Determination of presumptive vegetative compatibility groups of Verticillium dahliae occurring on sunflower using molecular markers

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Phytopathology 101:S241

Verticillium dahliae is a cosmopolitan asexual phytopathogenic fungus affecting more than 200 plant species, including sunflower (Helianthus annuus). Vegetative compatibility, the ability of an individual fungal strain to anastomose and form a stable heterokaryon, serves as the method for genetic exchange of this fungus. V. dahliae vegetative compatibility groups (VCG’s) vary in their level of pathogenicity and aggressiveness on different hosts. Verticillium wilt of sunflower was managed by the dominant gene V1 for the last two decades. However, a new proposed strain of V. dahliae (NA-Vd2), reported in Minnesota, North Dakota, and Manitoba, was found to be virulent on the V1 resistance gene. Despite the importance of VCG’s, reported VCG characterization of V. dahliae isolates collected from commercial sunflower has been limited to ten isolates, all of which originated from one region in Canada. The objective of this study is to characterize V. dahliae isolates into their presumptive VCGs on sunflower using amplified fragment length polymorphism (AFLP) markers. A total of 72 V. dahliae isolates from different locations and hosts and 30 tester isolates representing the different VCG’s were cultured, lyophilized, and DNA extracted. Eight primer pairs were used to verify the identity of the fungus as well as to identify the PCR

Genetic diversity of a global population of Colletotrichum coccodes using amplified fragment length polymorphism markers

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Phytopathology 101:S241

Colletotrichum coccodes (Wallr.) Hughes is an imperfect fungus in which vegetative compatibility may serve as a means of genetic exchange. Vegetative compatibility grouping (VCG) has been useful for measuring genotypic diversity but has certain limitations that reduce its effectiveness and utility. In the case of C. coccodes, isolates from different continents anastomose reliably and it is a useful tool in studying global population except for cases where no anastomosis occurs between nit mutants, or in case isolates are not capable to produce nit mutants. Molecular markers have proven very effective in studying the genetic diversity of several plant pathogens. The main objective of this study was to study the genetic diversity of a global population of C. coccodes, including North American, European/Israeli, Australian, and South African isolates, using AFLP markers. A total of 788 C. coccodes isolates were studied using three primer pairs. NA-VCG5 was the most common VCG globally, followed by NA-VCG2. Among the four regions studied, there was a relatively low gene diversity (h = 0.22), relatively high population differentiation GST (0.30), and low but significant linkage disequilibrium (rBarD < 0.09) except for isolates from South Africa. Overall gene flow (Nm) was 1.16, meaning that one or more individuals are exchanged among the five regions each generation. Most of the variation among the four geographic regions originated from the within population differentiation (7PT) (52.50%) while variation among regions and among populations was 0.00% and 0.05% respectively. Based on geographic origin, the global C. coccodes population had five main groups NA-VCG1, NA-VCG2, NA-VCG3, NA-VCG4/5, and NA-VCG6/7. Conversely, C. coccodes differentiation based on ancestry using a Bayesian clustering approach showed six main subpopulations. Values of gene diversity, population differentiation, linkage disequilibrium, gene flow, variation among subpopulations, and variation within subpopulations were 0.22, 0.46, 0.04–0.09, 0.58, 43%, and 57% respectively. Based on those analyses, the population of C. coccodes exists as one large population with four main groups (NA-VCG1/3; NA-VCG2; NA-VCG4/5; and NA-VCG6/7). Our data suggest that global C. coccodes population is closely related, and probably of the same origin. It is likely that C. coccodes became established on each continent as the potato was moved around the globe. Due to the lack of intermixing of the populations after their introduction, the C. coccodes population on a continent became bottle-necked which may explain the loss of vegetative compatibility among regional populations. AFLP, a dominant marker, proved valuable in differentiating and studying the global population of C. coccodes, however, using co-dominant markers which can detect heterozygous alleles, such as simple sequence repeat markers (SSR) could provide additional information at the genetic level and could further differentiate the global C. coccodes population.

Understanding the epidemiology of Cercospora kikuchii on soybean using foliar fungicides

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Phytopathology 101:S241

Foliar fungicides may be effective for managing Cercospora leaf blight (CLB) caused by Cercospora kikuchii in soybean. To improve our understanding of the epidemiology of C. kikuchii and to test the hypothesis that early foliar fungicide applications can disrupt the infection process of the pathogen, multifactorial trials were conducted in 2008 and 2009 at the Arlington and West Madison Agricultural Research Stations. Azoxystrobin + propiconazole (Quilt) was applied to four soybean varieties (AG2422, DSR221, DSR234, West Madison Agricultural Research Stations. Azoxystrobin + propiconazole (Quilt) was applied to four soybean varieties (AG2422, DSR221, DSR234, K245) at one of three growth stages (V5,R1,R3). Disease assessments were made one week post-R3 application on a weekly basis and yield was determined at harvest. CLB severity was numerically different among varieties in 2008, and was significantly affected by the variety x fungicide interaction in 2009 (P = 0.0024). The varieties AG2422 and K245 had higher severity in both years. There were no significant differences in CLB incidence and severity for the application timings, and in 2009 the highest severity was observed when Quilt was applied at V5 stage with the exception of the DSR221 variety. In 2008, there was no evidence of an effect of fungicide or variety on grain yield, while in 2009, there was an effect of variety (P = 0.0015). Current results suggest that early applications of azoxystrobin + propiconazole may not disrupt C. kikuchii infections in soybean and that further research is needed to investigate the role of latent infections.
Anthracnose stem blight, most often caused by Colletotrichum truncatum, has been documented to cause soybean yield loss and reduction in seed quality. There are several fungicides available in Iowa for use on soybean that includes anthracnose on their label. The effect of fungicides on anthracnose severity in five locations across Iowa in 2008 and 2009. Two fungicides, Headline and Stratego Pro, were applied at one of two timings (growth stages R1 and R3). Percent anthracnose severity was estimated on 20 plants from each plot at growth stage R8. Soybeans were harvested using a plot combine and all yields were standardized bushels per acre and at 13% moisture. Fungicides reduced anthracnose severity in both 2008 and 2009. However, there was no correlation between anthracnose severity and yield. Furthermore, yield component data was collected from 80 to 100 plants in the untreated control and correlated to the level of anthracnose on each plant. Data collected included pods per plant, seeds per pod, and seed size. There was no correlation between anthracnose severity and yield component data.

Evaluating the prevalence of bacterial blight of dry beans in North Dakota plant pathogen populations

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Phytopathology 101:S242

Bacterial blight has been a prevailing concern in most dry edible bean growing regions including North Dakota. The three major bacterial blight pathogens of dry beans reported in this area and their respective diseases are - Xanthomonas axonopodis pv. phaseoli (Xap) - Common bacterial blight; Pseudomonas syringae pv. phaseolicola (Psp) - halo blight and Pseudomonas syringae pv. syringae (Pss)- Bacterial brown spot. These are primarily seed transmitted and highly regulated, making them a major threat to the dry bean seed industry; particularly in North Dakota which is the largest producer of dry edibles in the country. The objectives of this study were to ascertain the prevalence of each of these three pathogens in North Dakota and to evaluate possible control. A foliar disease survey conducted in 2008 and 2009 covered 39 and 58 dry bean fields respectively. Pathogens isolated were identified through biochemical tests and species specific PCR. Psp was isolated from 47% and 12% of the fields surveyed in 2008 and 2009 and Pss was detected from 50% and 26% of the fields for the two years respectively. Pathogenicity tests were conducted on Pss and Psp isolates from 2009. All isolates were found to be capable of causing disease on a susceptible variety of dry bean but a variation in aggressiveness among Psp isolates was observed. Preliminary race typing results indicate the presence of race 6 and 8 of Psp in this region.

A survey of fungicide resistance in the Venturia inaequalis populations of Indiana and Michigan

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Phytopathology 101:S242

Venturia inaequalis, the causal agent of apple scab, infects apple trees. Long-term and extensive fungicide use has led to multiple fungicide resistances developing with varying frequencies in different orchards. To assess fungicide resistance levels, isolates of V. inaequalis were collected from Indiana and Michigan orchards. Single-spore derived isolates were evaluated using mycelium growth assays with previously determined baseline concentrations of fungicides and corresponding thresholds for growth. Fungicides tested include: kresoxim-methyl, thiophanate-methyl, dodine and myclobutanil. We identified isolates which were classified as resistant or shifting towards resistant to each fungicide. Resistance to kresoxim-methyl and myclobutanil, the primary fungicides used for apple scab management, was present in both states. This is the first report of field resistance to kresoxim-methyl in the United States. A total of 19% of isolates from Indiana and 44% in Michigan were shifted to kresoxim-methyl. Resistance to myclobutanil occurred in over 55% of isolates. Isolates that tested resistant or shifted often tested this way for multiple chemicals. Of 199 isolates tested, 38% were identified as resistant or shifted to two fungicides and 12% were resistant or shifted to all four of the fungicides. Statistical analyses of spore counts and radial mycelial growth resulted in no statistical differences between sensitive isolates and isolates resistant to one or more fungicides, suggesting no fitness penalty and yield with resistance based on these two parameters. The presence of resistance to all major fungicides used for apple scab management leaves growers with fewer control options. However, preliminary data indicates that the "second generation" DMI fungicide difenoconazole, when tested at the highest field rate, inhibits the growth of isolates resistant or shifted to a "first generation" DMI, such as myclobutanil. Thus, "second generation" DMIs may be a viable short-term management option for growers with DMI-resistance present in their orchards.

Response of Phytophthora root rot differential lines to Northern stem canker in soybean

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Phytopathology 101:S242

Northern stem canker (NSC), caused by Diaporthe phaseolorum var. caulivora (DPC), has re-emerged as a soybean disease of concern among farmers and crop consultants in the North Central region. Effective practices for managing risk from NSC have not been developed or critically assessed to date. Published, as well as unpublished, studies suggest that traditional approaches such as crop rotation or tillage changes, have little impact on controlling NSC. Therefore, availability of genetic resistance would be highly desirable for soybean producers wishing to reduce risk for losses from NSC. As part of a continuing series of studies on resistance to NSC, we screened the established set of Phytophthora root & stem rot differential lines for their reaction in greenhouse seedling inoculations. All fifteen lines were susceptible to infection by DPC, resulting in typical NSC symptoms, but there were quantitative differences in reaction. No differential resistance reactions were observed to NSC, but line L76-1988, carrying Rps2 was significantly less susceptible than other lines studied. Although resistance cannot be attributed to Rps2 based on this experiment, L76-1988 may nonetheless prove to be a useful source of quantitative or partial resistance to NSC.

Use of foliar fungicides for control of gray leaf spot of corn across environments

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One of the most damaging foliar diseases of corn in the north-central region is gray leaf spot (GLS), caused by Cercospora zeae-maydis (Czm). The availability of data from coordinated trials on the need for foliar fungicides to control GLS is limited. Trials were established in IA, IL, OH, and WI to examine corn hybrid and fungicide application timing effects for control of GLS. Using a split plot arrangement, the whole plot factor was corn hybrid and the subplot factor was fungicide active ingredient (non-treated, pyraclostrobin, or tetracazone) and timing. Four hybrids that differed in crop relative maturity and reaction to GLS were examined in each trial. Plants were inoculated using sterilized-soil infested with Czm from X9 to V12. Fungicide applications were made in 2008 at V12, VT, or R2, while in 2009, the timing was VT, R2, or R3. Trials were assessed for disease incidence and severity on the ear leaf as well as grain yield and grain moisture. Preliminary analyses have focused on individual trials. Results to date have indicated large differences in yield across environments. Only in trials from IL was there evidence of an effect of either hybrid (P < 0.0001 and P = 0.0034 in 2008 and 2009, respectively) or fungicide (P = 0.0019 in 2008 and 2009, respectively) on grain yield. Current results suggest that the risk of GLS differs across environments and impacts the likelihood that there is a yield response to fungicide.

Does previous crop history impact response to foliar fungicides in corn? P. D. ESKER (4), C. A. Bradley (3), P. Paul (2), A. Robertson (1)
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Phytopathology 101:S242

Results from foliar fungicide trials across the north-central region have not conclusively shown that previous crop affects yield response in corn. To improve understanding of the effect of previous crop and foliar fungicide timing, trials were conducted in IA, IL, OH, and WI in 2008 and 2009. In 2008, a preliminary trial was established with a single replication of previous crop (corn or soybean) in each state. Corn hybrids were selected for local environments and management for weeds and insects followed local recommendations. There were four fungicide treatments (timings): non-treated, V12, VT, or R2. The fungicide was a pre-mix combination of propiconazole + trifloxystrobin. Trials were assessed for foliar disease incidence and severity on the ear leaf as well as grain yield and grain moisture. Based on a multi-environment analysis, there was no evidence of a difference in grain yield or grain moisture among treatments. In 2009, trials were modified slightly by location, including use of single previous crop, replicated previous crop, and
multiple hybrids, and fungicide timing was also changed to VT, R2, or R3. Preliminary results for grain yield ranged from no effect of fungicide timing (IA), yield differences between previous crop soybean and corn (WI, \( P = 0.0572 \)), and yield differences between hybrid (IL, \( P = 0.0443 \)) and fungicide timing (IL, \( P = 0.0618 \)). Current results suggest that previous crop may not be a major predictor for grain yield response to foliar fungicides.

Widespread latent and pathogenic infection of soybean by the Phomopsis disease complex in Minnesota
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Top dieback and early-dying of soybean are common in August in Minnesota (MN). Occasional isolations suggested that the Phomopsis disease complex is often associated with these symptoms. This disease complex can cause significant damage to soybean. The goal of this study was to determine the distribution and potential importance of Phomopsis in MN soybean fields. For part A of this study, 70 symptomatic plants with top dieback, stem canker, and/or stem blight symptoms were collected from 50 fields in southern MN in 2006–2009. Fungi with morphological characteristics similar to Phomopsis and Diaporthe were isolated from stems. Genus was confirmed to be Diaporthe or Phomopsis using a standard PCR assay, and qPCR assays were used to identify species. ITS DNA sequences from a subset of the isolates all matched either \( P. longicolla \) or \( D. phaseolorum \) var. caulivora. \( P. longicolla \) was detected in 46% of the plant samples and \( D. phaseolorum \) var. caulivora was detected in 4% of the samples. For part B of this study, 150 asymptomatic plants were arbitrarily collected from 94 fields statewide in early August 2007, and the presence of these pathogens in stems was determined with PCR assays. \( P. longicolla \) was detected in 10% of the plants, and other unidentified Phomopsis species were detected in 35%. Further, common soybean cultivars were inoculated individually with \( P. longicolla \) or \( D. phaseolorum \) var. caulivora isolates using the cut stem inoculation method. All cultivars inoculated with either pathogen developed sunken, dark brown cankers; and leaves wilted and became necrotic at infected nodes. The results confirm that Phomopsis and Diaporthe are frequently associated with stem blight and top-dieback symptoms in MN, and these fungi are widespread as a group of latent and potentially damaging pathogens in asymptomatic plants.

Evaluation of fungicides and fungicide timing on management of sunflower rust (Puccinia helianthi) at three locations in North Dakota in 2008 and 2009
Phytopathology 101:S243

Sunflower rust, caused by Puccinia helianthi (Sch.), incidence has increased in U.S. sunflower fields since 2002. With little genetic resistance, fungicides may be management tool. However, the efficacy of many fungicides has not been assessed for sunflower rust management. Additionally, timing thresholds of newer fungicide chemistries (i.e. QoI) have been investigated. The objective of this study was to evaluate fungicide efficacy and timing for management of sunflower rust. In separate but adjacent experiments, fungicide efficacy (fungicide trial) and timing trials (fungicide trial) were arranged in a randomized complete block design. Trials were conducted in 2008 and 2009 at the North Dakota State University Research Extension Centers in Carrington, ND and Langdon, ND, and at a Cenex Harvest States research plot in southeast ND. Four row plots were sown at each location with the confection hybrid ‘Jaguar’. To facilitate disease development, spreader rows were inoculated with \( P. helianthi \) race 336 and supplemental irrigation was used as needed. In the fungicide trials, six to eleven fungicides were applied at R5. In timing trials, pyraclostrobin and tebuconazole were applied at R3, R5, and R6, singularly or sequentially. To evaluate disease, pustule coverage on the top four fully-expanded leaves of ten randomly selected sunflower plants was visually estimated with the aid of disease assessment diagrams. Evaluations were conducted three to six times and area under disease progress curve (AUDPC) values were generated. Results from the fungicide trials showed all fungicides reduced AUDPC from that of the non-treated control. Results from the timing trials indicated the most efficacious time to make a fungicide application was at R5.2 when disease severity was at 1–3%. Our study suggests that fungicide applications are an effective management tool for rust. However, timing of application should be further investigated.

Soil and rhizosphere populations of Fusarium and fluorescent Pseudomonas spp. associated with field-grown plants are affected by sorghum genotype
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Sorghum [Sorghum bicolor (L.) Moench] is valued for bioenergy, feed and feedlot. Potential of sorghum genotypes to support differing populations of root- and soil-associated fluorescent Pseudomonas spp. or Fusarium spp., in two soils, was assessed. Pseudomonad and Fusarium numbers were assessed from roots and soil of field-grown sorghum genotypes, RTx433 and Redlan. Possible biological control capabilities of Pseudomonas isolates, including hydrogen cyanide (HCN) and 2,4-diacetylphloroglucinol (ph) production, also were assessed. In dryland field conditions, RTx433 roots had greater numbers of pseudomonads than Redlan before and numbers increased over 3 weeks after. There were no differences in numbers of pseudomonads from dryland soil or roots or soil of irrigated plants. Percentages of HCN-producing root isolates and ph soil isolates declined on irrigated Redlan plants, but percentages of HCN-producers increased in dryland conditions. Redlan roots had greater percentages of Fusarium isolates in the Gibberella fujikuroi species complex. Results indicated that sorghum genotype affected rhizosphere populations of fluorescent Pseudomonas spp. and Fusarium spp. across soil environments.

Management strategies for stem rust (Puccinia graminis f. sp. tritici) of wheat using fungicides
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Phytopathology 101:S243

Stem rust, caused by Puccinia graminis f. sp. tritici (Pgt), is one of most devastating diseases of wheat throughout the world and is a growing threat to all wheat growing regions. The management of stem rust with fungicides is of particular interest due to the evolution of highly virulent races of Pgt in eastern Africa (e.g. Ug99). Herein we present results from studies evaluating fungicial activity against Pgt. First, a laboratory experiment was conducted to evaluate inhibition of spore germination using seven different chemicals. EC50 values were calculated and it was found that strobilurins were the best at inhibiting urediospore germination followed by the newer triazoles. Second, application timing was evaluated in the greenhouse with fungicides applied 24 hr, 48 hr, 96 hr and 168 hr before or after inoculation. All of the pre-inoculation treatments inhibited disease development; however the triazoles were significantly better when applied after infection. Field experiments were conducted in 2008 and 2009 using commercial products. In 2008, all treatments had lower stem rust severity and higher yields in comparison to the untreated control. Tebuconazole and prothioconazole were most effective in controlling stem rust. In 2009, no significant differences were observed, however disease development was limited on the untreated plots due to non-conducive weather conditions.

Genetic structure of Mycosphaerella graminicola populations in the major wheat-growing regions of the United States
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SCEPTOR tritici blotch (STB), caused by Mycosphaerella graminicola, is one of the major diseases of wheat worldwide. However, there is little information available on the population genetic structure of this pathogen in the major wheat-growing regions of the United States. We analyzed the genetic structure of 334 isolates of M. graminicola from 11 populations, three from spring wheat in California and North Dakota and eight from winter wheat in Indiana and Kansas using 17 microsatellite markers and two mating type loci. Clone-corrected data revealed that most of the M. graminicola populations had high levels of gene diversity (\( H = 0.31 \) to 0.52) and genotype diversity (GD = 0.98 to 1). Both the gene \( (H) \) and genotype diversity (GD) was higher for both populations from Indiana and North Dakota than California and Kansas. Similarly, there was a high level of gene flow (\( Nm = 1.73 \) to 23.30) and very less genetic distance (\( D = 0.52 \) to 0.98) among populations. Equal frequencies of mating types (MAT1-1 and MAT1-2) were found in all populations except in the California population. No evidence of linkage disequilibrium (LD) was observed in all populations. Overall, these results suggest that there is frequent sexual recombination in the M. graminicola populations and the populations are likely a single large population of M. graminicola affecting wheat fields in the United States.
Archaeophytopathology of global soybean rust
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Phytopathology 101:S244

Phakopsora pachyrhizi and P. meioliae are two rust species that infect soybean (Glycine max). A number of other hosts support the ureidinal growth of these Phakopsora, including Pachyzythrus erosus, Pueraria lobata, and Vigna unguiculata, but no aerial host is known. Traditionally, these two species vary in geographic distribution, with P. pachyrhizi confined to Asia, Africa & Australia, and P. meioliae confined to South & Central America. Several herbaria have accessions reported to contain one of the two species and include specimens from those locations, some nearly or over 100 years old. We sampled 38 of these archival specimens, and extracted & speculated the DNA of the fungus, if present, using quantitative PCR specific to P. pachyrhizi, P. meioliae, or to a third group inclusive of many rust species. Of the archival specimens, 11 were positive for P. pachyrhizi, including a 1912 specimen from Japan, but no P. pachyrhizi was found in specimens from before 1994 outside of Asia or Australia. Fifteen specimens were positive for P. meioliae, including a 1928 specimen from Brazil and two 1923 specimens from the Philippines. Twelve specimens (including all African accessions) were found to be negative for both species, but six were positive in the more inclusive rust assay, and included specimens from Tanzania, Sao Tome, Nigeria, Sierra Leone, and China; all had been labeled as P. pachyrhizi and none were on G. max. These results demonstrate the feasibility of DNA genotyping in archaeophytopathological investigations and suggest that P. pachyrhizi may have been more recently introduced to Africa than previously believed.

Quick identification of Xanthomonas campestris pv. translucens, Bacterial leaf streak causing pathogen of small grains
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In recent years, Bacterial leaf streak (BLS), caused by Xanthomonas campestris pv. translucens (Xct) has emerged as a potential threat to spring wheat production in the Northern Great Plains. Water-soaked, chlorotic or necrotic stripes on leaves are the characteristic symptom of the disease; however diagnosis based on field symptoms is not always accurate. Pathogen characterization and identification through conventional techniques is often time consuming, costly and inaccurate. Here, we examined application of the Biolog micro-plate system (Biolog, Inc., Hayward, Calif.) to identify the pathogen. Fifty bacterial strains were isolated from symptomatic wheat leaf samples in the field and subsequently identified using the Biolog micro-plate system. Results showed that the Biolog system was efficient in identifying the pathogen after inoculation. Those isolate caused water soaking in wheat seedlings with in 4 days after inoculation. Those other strains did not produce disease symptoms. Bacteria were re-isolated and rest were non-pathogens. The isolates identified as Xct produced bacterial leaf streak symptoms in pathogenicity tests while the other strains did not produce disease symptoms. Bacteria were re-isolated from symptomatic tissue to identify Koch’s postulates. Each of the three Xct isolates identified initially by the Biolog System were validated as Xct following inoculation, re-isolation and re-testing. Each Xct isolate caused water soaking in wheat seedlings within 4 days after inoculation. Those lesions were covered with bacterial ooze and bordered by a yellow margin. They were produced by yellow-brown longitudinal streaks within a week after inoculation. Results showed that the Biolog system was efficient in identifying the pathogen among numerous isolates. It can be combined with few conventional tests for preliminary identification and elimination of non-pathogens. Biolog system combining with limited conventional tests could be a less costly and more rapid technique in identifying plant pathogenic bacteria.

An Indiana survey of Phytophthora species in nurseries, greenhouses, and landscape plantings
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Phytopathology 101:S244

From 2006 to 2008, samples with symptoms consistent with Phytophthora blight and crown rot were collected as part of the USDA-APHIS Phytophthora ramorum survey and were supplemented by additional samples collected at commercial nurseries and retail nurseries. From 22 sites, 93 Phytophthora isolates were obtained from 1657 host samples containing 15 plant genera. Comparison of the internal transcribed spacer (ITS) sequence of the ribosomal DNA identified 10 Phytophthora sp. A majority of the isolates were either P. citricola (39.8%) or P. citrophthora (28.6%). P. citricola isolates were collected at 12 sites from 5 host genera, and included Forsythia, Juglans, Pieris, Rhododendron, and Syringa. P. citrophthora was isolated from 11 sites on 7 host genera: Calycanthus, Forsythia, Ilex, Kalina, Pieris, Rhododendron, and Syringa. The other identified Phytophthora sp. consisted of P. cactorum, P. cactorum x hedraandra, P. cambivora, P. capsici, P. cryptogea, P. drechsleri, P. nicotianae, and P. syringae. Sixteen isolates showed signs of possible species hybridization. Four isolates were found to be hybrids of P. cactorum and P. hedraandra as verified by cloning and sequencing the ITS regions. Three of the P. cactorum x hedraandra isolates came from Rhododendron plants at the same site. The other hybrid isolate was recovered from Dicentra, which is not a known host of either of the parental species, P. cactorum or P. hedraandra, and suggests an increase of host range due to species hybridization.

Inoculation timing, mist duration and isolate effects on Fusarium head blight and deoxynivalenol in two hard red spring wheat cultivars
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The effects of inoculation timing, mist duration, and the Fusarium graminearum isolate’s trichothecene genotype on Fusarium head blight (FHB) severity and deoxynivalenol (DON) content in grain were evaluated in a greenhouse on two spring wheat cultivars (Glenn, rated MR to FHB, and Trooper, rated MS to FHB). Growth stages at inoculation timing were early flowering (Feekes 10.51), kernel watery ripe (Feekes 10.54) or kernel medium to late milk (Feekes 11.1). Spores from North Dakota isolates of a 3DON or a 15DON trichothecene genotype were atomized onto wheat heads. Following inoculation, plants were placed in a mist chamber for two, five or 10 days, and then were moved to a greenhouse bench. Each treatment had 10 plants per replicate, four replicates per treatment, and each trial was repeated four times. Plants were stored in plastic bags for 4 days post inoculation for disease FHB index (25% inoculum head severity)/100. At maturity, all inoculated heads were harvested and grain was ground and analyzed for DON content using gas chromatography and electron capture. Results indicated that the highest DON level occurred with the 3DON inoculation of Trooper wheat, when analyzed across all mist and growth stage treatments. The 10 day mist period resulted in the highest FHB index and DON production, regardless of isolate, growth stage or cultivar used. The Feekes 10.51 and 10.54 inoculations with the 3DON isolate did not differ significantly in FHB index or DON production, but the Feekes 10.54 inoculations with the 15DON isolate resulted in higher FHB and DON than Feekes 10.51 inoculations. The late inoculations, at Feekes 11.1 growth stage, did result in FHB infection, but had the lowest FHB and DON among growth stage inoculations, over all mist durations, isolates and cultivar treatments.

Corn ear molds and mycotoxins in North Dakota, 2009
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Phytopathology 101:S244

In 2009, North Dakota grew 2 million acres of corn. Temperatures in 2009 averaged below normal across the state during the growing season, with accumulated corn growing degree units ranging from 0 to 180 units below normal. A killing frost in the second week of October and subsequent cold temperatures and rains contributed to high moisture corn and development of corn ear molds. NDSU coordinated a corn mold survey in response to grower concerns about corn ear molds. County and area extension specialists submitted samples of 6-12 ears per field from fields in 24 counties representing major corn growing areas. A total of 94 corn samples from the NDSU extension survey effort and private submissions were evaluated for molds and mycotoxins. The predominant fungus identified via microscopy was Cladosporium species, but some samples also had visible hyaline or pink hyphae, with some of these confirmed to be species of Fusarium. The 94 samples were screened by the NDSU Veterinary Diagnostic Laboratory for 17 trichothecenes (mycotoxins produced by Fusarium species), using gas chromatography/mass spectrometry techniques. Aflatoxin was not detected in 73% of samples. Of the remaining 27% with detectable mycotoxins, deoxynivalenol (vomitoxin) was predominant (60% of positive samples). However, only 4 of the 15 samples positive for deoxynivalenol had levels greater than 1 ppm. Additional mycotoxins detected included zearalenone (in 3 samples); T-2 toxins (in 10 samples); and nivalenol (in 3 samples).

Genetic and morphologic diversity of Streptomyces species that cause potato common scab in Michigan
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Potato common scab is a worldwide disease caused by several Streptomyces spp. In Michigan (MI), Streptomyces scabies is the predominant pathogenic species.
species. A survey was conducted to analyze the distribution and diversity of Streptomyces species in MI. Potato tubers with typical common scab symptoms were collected from various locations. One hundred and fifty isolates of Streptomyces were isolated from tuber lesions and purified by transferring single colonies on Streptomyces selective media. Morphology of the isolates was examined on different International Streptomyces Project media. To date, 49 isolates have been identified as Streptomyces and processed as follows. Pathogenicity of the isolates was determined in the greenhouse using potato plants grown from seed tubers. Soils were inoculated with individual isolates and common scab assessed on daughter tubers using a categorical scale (0–5 where 0 = no scab symptoms and 5 = pitted scab on >50% of the tuber surface). Genomic DNA was extracted from the isolates and polymerase chain reaction (PCR) was conducted using primers of marker genes such as txtAB, TomA, and nec1 for pathogenic Streptomyces spp. Gene of 16S rRNA was amplified, sequenced and analyzed for each isolate using the BLAST algorithm against the NCBI Genbank. Some of the sequences have been submitted to the GenBank. The capability of isolates to produce thaxtomin was determined from oat brain medium culture and confirmed with high performance liquid chromatography. Four isolates were S. stellascabiei, six were S. scabies, and the remaining 39 were classified as Streptomyces species. This is the first report of S. stellascabiei in MI. All 49 isolates were pathogenic and txtAB, nec1, and TomA were present in all isolates except 3 of the isolates (DS21, KRUF21, and LENO2) that lacked either nec1 or tomA. Overall, the Streptomyces spp. isolates examined were phenotypically and genetically heterogeneous, indicating diversity.

Ascospore germination and infection efficiency of Sclerotinia sclerotiorum in relation to temperature

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Phytopathology 101:S245

The effect of constant temperature on S. sclerotiorum ascospore germination and its infection efficiency were assessed on canola plants in controlled environment. The germination study had three replications and five temperatures (10–35°C, at 5°C intervals). For every temperature, 20 µl of a suspension from a mix of the three isolates was inoculated on 60 canola plants. For every temperature, isolates, and ascospore concentrations. A 20 µl ascospore suspension from a mix of the three isolates was inoculated on 60 canola petioles. Inoculated petioles were placed on leaves of three plants per rep and incubated for at least 10 days. Plants were misted periodically to promote infection. Infection efficiency was assessed by calculating the percentage of petioles producing Conidiophore (Cm) or measuring lesion size every two days after inoculation (DAI). Optimum conditions for ascospore germination and growth occurred between 20 and 25°C. After 24 hrs of incubation at these conditions, germination was 77% and germ tubes were 31 µm long. Further, 50% of ascospores germinated within two hrs and had germ tubes 7 µm in length. First disease symptoms were observed within four and nine DAI on plants inoculated at 20°C and 10°C, respectively. The fungus destroyed the entire leaves within nine and twenty DAI when incubated at 20°C and 10°C, respectively. No symptoms developed at 30°C.

Uniform fungicide timing and efficacy trials for management of common bean rust in North Dakota

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Phytopathology 101:S245

The most recent common bean rust (Uromyces appendiculatusPers.:Pers.) epidemics in North Dakota’s dry edible bean (Phaseolus vulgaris L.) crop caused an excess of $10 million in losses in 1994 and 1996. Rust has been managed with the resistance gene Ur-3, which was effective to all then-known races of rust in North Dakota, and widely deployed in commonly used cultivars. In 2008, a new race of rust conferring virulence to Ur-3 was detected for the first time in North Dakota, rendering the North Dakota crop susceptible to rust. Fungicides are a potential management tool for rust, however, many fungicides have not been evaluated for efficacy to identify the most effective application timing on bean rust. The objective of this research was to evaluate the efficacy and timing of fungicides for rust management. Two separate experiments were planted to the pinto bean cultivar GTS-900, which lacks the Ur-3 gene, in RCB designs at three North Dakota locations in 2009. To facilitate disease development, plots were inoculated with ureidiospores of an U. appendiculatus isolate collected in 1996 known to be avirulent to Ur-3 and virulent on GTS-900. Disease was evaluated by visually estimating pustule coverage from a sample of ten leaves per plot. Plots were rated 4 to 6 times and Area Under Disease Progress Curve (AUDPC) was calculated. Results indicate that 1) most fungicides reduced AUDPC values compared to the check; 2) timing of these fungicides is a critical factor in reducing disease levels and increasing yield, and 3) the greatest reduction in AUDPC occurred with sequential applications.

Effect of multiple Ceratocystis smalleyi infections on stem water conductance in maturing bitternut hickory

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Phytopathology 101:S245

Hundreds of cankers caused by Ceratocystis smalleyi are associated with declining, bark beetle-attacked bitternut hickory in the northeastern and north central U.S. Sapwood infections associated with the cankers are hypothesized to disrupt xylem water transport and contribute to crown wilt and tree death. In a field study, stems of healthy bitternut hickory (12.6 to 23.4 cm diameter at 1.4 m) were inoculated at 50 points (between 1.8 and 3.7 m stem height) with isolates of C. smalleyi or with sterile water in July 2008. In September 2009, sap flow in the study trees was monitored for 18 days using heat dissipation sensors. Although no crown wilt symptoms were observed, cankers were commonly found around inoculation points on the fungus-inoculated trees. All trees were felled, and measurements were made of inner bark diameter, sapwood discoloration, and several xylem vessel features. Infected trees exhibited reductions in sap flux and daily water loss compared to controls (water inoculated and non-inoculated trees). Daily water loss for representative days was inversely correlated with the relative proportion of cankered bark area and numbers of tyloses in the outer two annual rings of the study trees. Measurements of other vessel features did not differ between infected and control trees. Preliminary results suggest multiple stem infections of C. smalleyi impair water transport in otherwise healthy bitternut hickory. A replicate field experiment is underway.

Relative aggressiveness of Contans WG® and native Coniothyrium minitans isolates from Wisconsin soybean fields

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Phytopathology 101:S245

Sclerotinia stem rot (SSR) of soybean, caused by Sclerotinia sclerotiorum, can cause large yield loss in soybean, particularly in Wisconsin. Crop rotation is not used to control of SSR, as sclerotia of Ss are long lived in the soil. Coniothyrium minitans (Cm) parasitizes Ss and a commercial formulation of Cm, Contans WG, has been approved for use on soybean. It is not known how well the Contans WG isolate can compete with native Cm populations. Experiments were designed to compare native and Contans WG isolate aggressiveness. Cm was isolated from soil from the Marshfield (Isolates 1 and 2) and Arlington (Isolates 3 and 4) Agricultural Research Stations in WI. Treatments in Trial 1 included isolates 2 and 4, Contans WG and a negative control, while Trial 2 included isolates 1 and 3, Contans WG and a negative control; each treatment was replicated four times. For each trial, sclerotia were individually inoculated on 10 µL of a 2 × 10 6/mL spore suspension or water and incubated in a petri dish in moist, sterile soil. After 14 days, 10 sclerotia were removed, surface sterilized, halved, and plated onto amended PDA. After 14 days, sclerotia were rated for Cm colonization. Contans WG produced more spores on colonized sclerotia than Isolate 3 (P = 0.01), but Contans WG produced more spores on colonized sclerotia than Isolate 3 (P < 0.0001). Implications for the biocontrol potential of Contans WG in WI are discussed.

Method and timing of azoxystrobin application to control Rhizoctonia root rot of sugar beet

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Phytopathology 101:S245

Rhizoctonia solani (Kühn) is the causal agent of Rhizoctonia root rot of sugar beet (Beta vulgaris L.) in North Dakota (ND) and Minnesota (MN). Studies were conducted to (i) determine the best method of applying azoxystrobin for controlling R. solani and (ii) determine the effect of azoxystrobin on disease control when applied pre- and post inoculation. Sugar beet plants were inoculated at the four leaf stage and fungicides were applied subsequently as a hypocotyl drench or banded. Hypocotyl drench gave similar control as the
banded application. To determine the best time to apply azoxystrobin, hypocotyls were drenched at the four-leaf stage at 0, 3, 10, 14, and 21 days post-inoculation and 0, 7, 14, and 28 days pre-inoculation. Azoxystrobin was not effective at controlling Rhizoctonia root rot when applied after inoculation. Azoxystrobin when applied prior to or on the day of inoculation provided effective Rhizoctonia root rot control.

**Depth at which Rhizoctonia solani causes infection of sugar beet**

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Phytopathology 101:S246

*Rhizoctonia solani* (Kühn) is the causal agent of Rhizoctonia root rot of sugar beet (*Beta vulgaris* L.). Typically, *Rhizoctonia* root rot symptoms appear to be initiated on the plant at the soil line. Recently, sugar beet plants were observed with *Rhizoctonia* root rot infections close to the root tips and *R. solani* AG 2-2 IIIB was identified as the causal agent. There were concerns that AG 2-2 IIIB may be causing infections lower on the roots which may make fungicide control difficult. Our objective was to determine the depth which is most favorable for infection. Plants were inoculated at the four-leaf stage using two grains of barley inoculated with *R. solani* AG 2-2 IIIB. Inoculum was buried at 2.54, 7.62, and 12.70 cm depth. Plants were rated for root rot disease severity using the 0–7 rating scale fourteen days after inoculation. Results indicated that infections by *R. solani* AG 2-2 IIIB occurred at all depths. However, *R. solani* appears to favor the area on the roots just below the soil line irrespective of the depth of placement.

**Characterizing Phytophthora infestans US22 for fungicide resistance, and pathogenicity and virulence on tomato cultivars**

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Phytopathology 101:S246

Late blight of tomato and potato, caused by the oomyceteous pathogen Phytophthora infestans (Mont.) deBary, is one of the most devastating plant diseases worldwide. In 2009, a new type of *P. infestans* was identified in multiple U.S. states, primarily on tomato, and has been designated as US22. Fourteen isolates of *P. infestans* were collected from tomato and potato throughout Wisconsin during the 2009 growing season. Each isolate was characterized for resistance to the fungicides metalaxyl and metalaxyl-A. All isolates exhibited sensitivity to 100 ppm metalaxyl and 100 ppm of the enantiomer metalaxyl in amended rye agar. Fifteen commercially available tomato cultivars with supposed resistance to late blight were screened for foliar resistance with a sporangial suspension of a single zoospore derived isolate of *P. infestans* collected from tomato in southern Wisconsin. Detached leaves of ‘Matt’s Wild Cherry’ and ‘Wapsipinicon’ were fully resistant at 9 days post inoculation (dpi). Thirteen of the tested cultivars were susceptible and exhibited pathogen growth and water soaked lesions as early as 5 dpi. Several cultivars exhibited a hypersensitive response (HR) at the site of inoculation, with ‘Pruden’s Purple’ exhibiting the most dramatic and consistent response. Our findings indicate the potential utility of metalaxyl or metalaxyl-A fungicides and tomato varietal resistance for the control of this new *P. infestans* type.

**Is *Avr1a* gene present in virulent isolates of *Phytophthora sojae* from Iowa?**

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(1) Iowa State University

Phytopathology 101:S246

*Phytophthora sojae* races or pathotypes are defined by their ability to infect a set of differential soybean cultivars that carry particular *Rps* resistant genes. Avirulence gene *Avr1a* of *P. sojae* determines the outcome of the interaction with the resistance *Rps*-la gene in soybean. *Avr1a* has been identified as encoding an RXLR effector that is expressed in avirulent pathotypes but not in virulent ones (1). Expression of the *Avr1a* was congruent with avirulence-

**Comparison of inoculation methods for evaluating root pathogens of wheat**

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(1) South Dakota State University

Phytopathology 101:S246

Greenhouse experiment was conducted to compare inoculation methods for common root and crown rot, caused by the fungus *Cochliobolus sativus* and *Gibberella zea* respectively against six varieties each for spring and winter wheat. Two inoculation methods used were: 1) mixing inoculated millet and potting mix in the ratio of 1:50 at planting, and 2) dispensing the 10 µl/pot of *Cochliobolus* (104/ml) and *Gibberella* conidial suspension (105/ml) directly at plant roots, 15 days after planting in spring wheat and 57 days (after vernalization) in winter wheat. The winter wheat was vernalized for 42 days at two-leaf stage. Plant height and percent severity of the sub crown internode (SCI) were assessed at 35 days after inoculation in both spring and winter wheat for five plants/pot and dry weight was taken. Disease data was analyzed using analysis of variance (ANOVA). Results indicate that height and dry matter weight were significantly higher in inoculated plants than those in uninoculated plants for both inoculation methods and fungi (P-value < 0.001). It should be noted that no sterile millet was added to the control pots. However, mixing of inoculum with potting mix had significantly higher SCI symptoms than applying suspension inoculum on roots (P-value < 0.001).

**Effect of fungicide seed treatments on Fusarium virguliforme and Sudden death syndrome of soybean**


(1) Southern Illinois University; (2) USDA; (3) University of Illinois

Phytopathology 101:S246

Sudden death syndrome (SDS) is a yield reducing disease increasing in prevalence across soybean producing states. Recent research indicates the SDS pathogen, *Fusarium virguliforme*, can infect as early as initial radicle emergence. This suggests fungicide seed treatments could offer some protection against *F. virguliforme* during early soybean development. In 2008 and 2009 field studies across two locations and a greenhouse study were conducted to evaluate eleven fungicide seed treatments and a non-treated control across moderately resistant and susceptible cultivars for effects on *F. virguliforme* DNA concentrations present in V1 taproot samples. Root samples collected at three times during the season were digitally scanned and analyzed using specialized software (WinRHIZO) to measure *F. virguliforme* DNA concentrations present in V1 taproot samples. Root samples collected at three times during the season were digitally scanned and analyzed using specialized software (WinRHIZO) to measure *F. virguliforme* DNA concentrations present in V1 taproot samples. Root samples collected at three times during the season were digitally scanned and analyzed using specialized software (WinRHIZO) to measure *F. virguliforme* DNA concentrations present in V1 taproot samples. Root samples collected at three times during the season were digitally scanned and analyzed using specialized software (WinRHIZO) to measure *F. virguliforme* DNA concentrations present in V1 taproot samples. Root samples collected at three times during the season were digitally scanned and analyzed using specialized software (WinRHIZO) to measure *F. virguliforme* DNA concentrations present in V1 taproot samples.
2010 Pacific Division Meeting Abstracts

Abstracts presented at the joint meeting of the APS Pacific Division and the Canadian Phytopathological Society in Vancouver, British Columbia Canada, June 20–23, 2010. The abstracts are arranged alphabetically, by first author’s name.

Potential for forecasting late blight on specialty potatoes in western Washington of the U.S.
G. Babette (1), D. A. INGLIS (1)
(1) Washington State University Mount Vernon NWREC Phytopathology 101:S247

This study focused on adapting a disease forecasting system called WISDOM (University of Wisconsin) for predicting late blight epidemics in specialty potato production systems of western Washington (WWA). Although disease forecasting is generally known to reduce unnecessary fungicide sprays and application costs in crop production, none have been developed with potato late blight, potato seed piece inoculum, and WWA deliberately in mind. Using 12 years of historical environmental and late blight data accumulated at WSU-NWREC in Mount Vernon, WISDOM correctly predicted late blight lesion onset 58% (7 out of 12 years) and late blight lesion spread 92% (11 out of 12 years) of the time. WISDOM-predicted fungicide spray intervals ranged between 5 and 10 days. During low disease pressure years (1996, 2001, 2003, 2009) WISDOM advised 16 fewer sprays while during medium-to-high disease pressure years (1994, 1995, 1997, 1999, 2000, 2002, 2008) WISDOM advised applying 3 more sprays than used in WSU potato research plots. Savings in fungicide costs and quantities in WWA will most likely be realized from eliminating unnecessary late blight sprays in a low disease pressure year rather than in a medium or high disease pressure year. However to use late blight forecasting successfully in WWA, it must be integrated with (i) routine late blight seed piece fungicide treatment, (ii) a calendar foliar fungicide spray at crop green-row stage, and (iii) comprehensive late blight sanitation practices.

Quantitative and qualitative variations in expression of pathogenicity genes of Pythium aphanidermatum during root rot infection of different hosts
(1) Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, CANADA; (2) Dept. of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, U.S.A.; (3) Dept. of Plant Biology, Michigan State University, East Lansing, MI, U.S.A.; (4) Forensic Science Program, Trent University, Peterborough & Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, CANADA; (5) Forensic Science and Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON, K9J 7B8, CANADA Phytopathology 101:S247

Pythium aphanidermatum is a major pathogen of greenhouse vegetables and several other economically important crops. To investigate differences in gene expression during infection of different hosts, we are planning to analyze the transriptome of P. aphanidermatum at different time points during disease development in seedlings of monocot and dicot crops. The annotated genome of P. ultimum var. ultimum DAOM BR144 (43Mb) and the assembled but unannotated genome of P. aphanidermatum have been used to first investigate the putative secretome of P. aphanidermatum. Similar to P. ultimum, P. aphanidermatum does not seem to have the classical RXLR effector proteins. However, it does contain necrosis inducing proteins, serine and cysteine pro tease inhibitors, various proteases, as well as CBEL-like and ras-like proteins. Reverse transcriptase assays will be developed for some of the genes coding these proteins to establish the optimum treatments and timings for extraction of RNA to study host pathogen interaction differentials. Gene expression profiles under different host crops during root rot disease development will be investigated with next generation sequencing technology. Data from these studies will be also used to annotate the genome of P. aphanidermatum.

Cross-pathogenicity of Verticillium dahliae isolates from skullcap and peppermint
J. K. DUNG (2), L. J. du Toit (1), E. W. Gatch (1), D. A. Johnson (2)

Skullcap (Scutellaria lateriflora, Lamiaceae) is a herb used in alternative medicine. A commercial skullcap crop with foci of wilted and necrotic plants was examined in Washington in 2008. Three fungal isolates from plants were identified as Verticillium dahliae (Vd) based on morphology and a Vd-specific PCR assay. Skullcap and peppermint (Mentha x piperita) cultivars ‘Black Mitcham’ (susceptible to Verticillium wilt) and ‘Redefined Murray’ (moderately resistant) were inoculated with a peppermint, a potato, and three skullcap isolates using a soil drench (10^6 conidia/cm^3). Disease ratings were converted to area under disease progress curves (AUDPC), and aboveground biomass was recorded. All skullcap isolates caused typical Verticillium wilt symptoms (chlorosis, wilting and necrosis) on skullcap and ‘Black Mitcham’. Isolates from skullcap and peppermint caused significantly greater (P < 0.05) AUDPC values and reduced yields on skullcap and ‘Black Mitcham’ compared to the potato isolate. One skullcap isolate caused significantly greater AUDPC values on ‘Redefined Murray’ than the other isolates. The potato isolate caused mild or no symptoms on all hosts. V. dahliae was observed on 5% of seeds harvested from skullcap plants inoculated with the mint isolate, but 0% of seeds from plants inoculated with the other isolates. This is the first report of Vd infecting skullcap, cross-pathogenicity among isolates from skullcap and peppermint, and seedborne Vd in skullcap.

A community-based stream monitoring program in western Washington for early detection of invasive Phytophthora spp.
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(1) Puyallup Research and Extension Center, Washington State University, Puyallup, WA, U.S.A. Phytopathology 101:S247

To supplement state agencies in their monitoring for Phytophthora ramorum, the sudden oak death (SOD) pathogen, a community-based stream monitoring program was begun in 2010. This project will expand on the streams currently being sampled by the WA Dept. of Natural Resources (WADNR) as part of the national P. ramorum survey and on nursery surveys by WA State Department of Agriculture (WSDA) and will allow for early detection of P. ramorum and other invasive Phytophthora species, as well as examining the biodiversity of Phytophthora spp. in stream ecosystems. Sites were chosen based on input from WSDA and WADNR and on volunteer availability. The baiting process involves placing Rhododendron ‘Nova Zembla’ leaves in mesh bags and deploying them in the stream for two weeks. Four sites are...
being monitored for six intervals and three sites for one two-week baiting period. After bait retrieval the leaves are cultured on Phytophthora-selective media and colonies of Phytophthora are isolated onto V8 agar. Phytophthora species are identified using molecular and cultural methods. Volunteers consist of Master Gardeners, high school and college students, and others. In addition to baiting, some of the student groups are doing research projects on Phytophthora in the lab as part of their class requirements. More baiting sites are planned for 2011.

Further studies on zoospore germination inhibition of three lineages of Phytophthora ramorum by chemical fungicides, and identification of a potential bacterial biocontrol agent M. ELLIOTT (1), S. F. Shamoun (2), G. Sumampong (2), D. James (1), S. Masri (1), A. Varga (1)

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Phytopathology 101:S248

Thirteen fungicides with varying modes of action were evaluated for their effectiveness on inhibiting zoospore germination of Phytophthora ramorum. Nine isolates of P. ramorum representing the three clonal lineages were tested. During this study some of the isolates were found to have bacteria associated with the zoospores. We have observed that when bacterial contamination is present, very little or no sporangia are produced. Three morphologically different bacteria have been observed under the compound microscope from contaminated cultures. It is our intention to purify these three bacteria and identify them using the 16s rDNA sequence. The biological significance of these bacteria and their potential use as biocontrol agents will be explored. All of the fungicides were effective in preventing zoospore germination at the recommended doses for controlling Phytophthora disease on ornamental plants. EC50 values were lower for P. ramorum on many of the fungicides than for other Phytophthora spp. commonly found in agriculture, suggesting that resistance has not yet developed in this species. There was variability among isolates in their sensitivity to different chemicals. Use of some chemical fungicides and biological control agents can be an effective tool in managing P. ramorum in nurseries as part of an integrated pest management program.

Fungal species associated with coast live oak (Quercus agrifolia) mortality in Southern California A. ESKALEN (3), S. C. Lynch (1), P. Zambino (4), T. Scott (2)

(1) Center for Conservation Biology, University of California, Riverside; (2) Department of Earth Science, University of California, Riverside; (3) Department of Plant Pathology and Microbiology, University of California, Riverside; (4) USDA Forest Service, Pacific Southwest Region, San Bernardino, CA

Phytopathology 101:S248

Sharp decline and mortality of coast live oak (Quercus agrifolia) has been observed in San Diego County, California, since 2002. Much of this decline has been attributed to a new pest in California, the goldspotted oak borer (GSOB, Agrilus coxalis). Associated symptoms of crown thinning, bark cracking and/or peeling, patches of stain (1–10 cm diameter) and bleeding on the bole, and tree death have mostly been observed on individuals over 30 cm diameter at breast height (DBH). In 2008, a Botryosphaeria species was recovered from necrotic tissue of bleeding bole cankers from GSBG-affected trees in Jamul, San Diego County. Zone lines separated dead and live tissue in affected phloem and xylem. A survey of oak stands throughout San Diego and Riverside Counties in 2009–2010 consistently recovered three Botryosphaeria species along with Biscogniauxia mediterranea, Togninia fraxinipennylanica, Hypoxylon sp. Daldinia sp. Pezicula sp., Bionectria sp., Phialophora sp., Daldinia sp. and Daldinia sp. from the bark of bleeding trunk and branch cankers at all eight locations—those both within and extending to 20 miles beyond areas infested by GSBG, where tree mortality has been observed. Species identification was confirmed by ITS4/5 rDNA sequence comparisons in Genbank. Pathogenicity tests are underway.

Development of a soil bioassay to assess the relative risk of spinach Fusarium wilt with greenhouse soil bioassay to assess the relative risk of spinach Fusarium wilt for individual fields. Soils were sampled from three fields shown to differ in inoculum potential based on preliminary evaluation. Subsamples of each soil were pasteurized at three levels to obtain a range of inoculum densities. Three female spinach lines (highly susceptible, moderately susceptible, and moderately resistant to Fusarium wilt) were planted in each soil-pasteurization combination and rated weekly for Fusarium wilt severity. Soil, spinach line, and pasteurization treatments significantly affected disease severity and plant biomass. An index of wilt severity effectively differentiated the disease potential associated with each treatment. To test the bioassay further, growers submitted soil sampled from 26 fields intended for spinach seed production in 2010. Each soil and the appropriate soil control treatments were assayed using the same three spinach lines. Soil, spinach line, and the interaction between these factors significantly affected severity of Fusarium wilt, with disease ratings 28 and 35 days after planting providing optimum differentiation of Fusarium wilt risk among the soils. The accuracy of these wilt predictions will be determined by evaluating Fusarium wilt severity in spinach seed crops grown in these fields in 2010.

Development of a quantitative real-time PCR assay for assessment of Phytophthora rubi in soil J. A. GIGOT (1), T. Walters (1)

(1) Washington State University-NWREC Mount Vernon, WA, U.S.A.

Phytopathology 101:S248

Phytophthora rubi is a serious pathogen of raspberry in northwestern Washington. A real-time PCR assay was developed to quantify P. rubi inoculum in soil using primers and a TaqMan™ probe adapted from a previous publication. A standard curve was created for the TaqMan assay using P. rubi DNA amounts ranging from 1 ng to 1 fg. In order to relate DNA quantity from the standard curve to propagule density in soil, DNA was extracted from triplicate samples of sterile field soil infested with P. rubi oospores at densities of 0, 10, 100 and 1000 oospores/g soil. To assess the effect of these oospore densities of P. rubi on raspberry root rot, a greenhouse bioassay was developed. Raspberry tissue culture plants (cv. Meeker) were planted into sterile field soil infested with oospores (0, 10, 100 or 1000/g soil) and grown in conetainers for ~6 weeks (6 replications, 2 tests). Plants were analyzed for root rot disease on a 0 to 9 scale (0 = healthy, 9 = severe symptoms). Mean cycle threshold values for DNA extracted from infested field soil was significantly (P < 0.05) different between 1000 (28.8) versus 10 (31.2) and 0 (36.0) oospores/g soil. In the greenhouse bioassay, there were significant differences in root rot ratings among raspberry plants inoculated with 1000 (7.7), 10 (3.3) and 0 (0.5) oospores per gram of soil. Further work is needed to optimize DNA extraction efficiency from soil. This real-time assay will be useful in future studies on the impact of soil treatments with chemical fumigants or alternative practices on the fate of P. rubi inoculum in soil.

Purification and characterization of novel glucanases from Trichoderma harzianum ETS 323 S. LIU (1), H. Jhan (2), C. Chen (1), K. Peng (2)

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Phytopathology 101:S248

Trichoderma harzianum ETS 323 secretes at least two glucanases, a 23.5 kDa endo-β-1,3-glucanase (EG Th1) and a 60 kDa exo-β-1,3-glucanase (ExG Th1) that have important roles in biocontrol mechanism. Both the enzymes were identified by their reaction products and were purified to homogeneity. The temperature and pH optima for EG Th1 (7.3 fold purification, yield 5.0%) and ExG Th1 (33.7 fold purification, yield 0.15%) were 50°C and pH 4.5, respectively. Kinetic parameters of EG Th1 (Km: 25 mg mL⁻¹, Vmax: 294 μM min⁻¹, specific activity: 7.4 U mg⁻¹) and ExG Th1 (Km: 85 mg mL⁻¹, Vmax: 385 μM min⁻¹, specific activity: 24.6 U mg⁻¹) towards carboxymethyl cellulose (CMC) were determined. Both enzymes favored CMC and MnCl₂ over other substrates tested and maintained 100% activity for 10 days at 38°C. Metal ions such as KCl, MgCl₂, HgCl₂, and FeCl₃ showed approximately 30% inhibition against EG Th1 but not ExG Th1. Both enzymes catalyzed transglycosylation of glucose in the presence of cellulose; however, ExG Th1 exhibited better activity and higher product diversity.

Identification of expressed sequences in lily under pathogen attack and rhizobacterium induction Y. Liu (1), K. Yang (1), C. CHEN (1)

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Phytopathology 101:S248

There are evidences showing that Bacillus cereus effectively induces systemic resistance in Lilium formosanum and lily cv. Star Gazer. Differentially expressed genes of GRP1 (glycinin-rich protein), MT1 (metallothionein-like protein), and PsbR (photosystem II) had been identified by suppression
subtractive hybridization. Expression of these three genes in lily leaves increased in response to Botrytis ellipit-ca infection; but GRP1 and PsbR gene expressions decreased in the leaves of B. cereus-treated lily plants with or without subsequent B. ellipitica-infection. In a cDNA-AFLP (amplified fragment length polymorphism) analysis of B. ellipitica-infected leaves and the leaves of B. cereus treated lily plants with or without subsequent Botrytis inoculation, over 100 cDNAs were cloned without the electrophoresis step. Sequence analysis indicated that most of the cloned cDNAs were metabolism and chromosome-related, and some were signal transduction, cell defense, transport, energy, protein synthesis, and transcription-related. Among them, four were analyzed by semi-quantitative RT-PCR to show the differential expression patterns under conditions of pathogen attack, rhizobacterium treatment, and reduced systemic resistance. B. cereus-treatment and B. ellipitica-infection could increase gene expressions of GTase-binding protein, calmodulin, and glutamine synthetase; however, expressions of these genes gradually decreased after Botrytis inoculation on B. cereus-treated lily plants. Since gene expression of glutamine synthetase could be induced by abscisic acid but suppressed by salicylic acid, and the fact that B. cereus-treatment suppressed gene expression of pathogenesis-related protein 1, it is presumed that the signaling pathway of B. cereus-induced systemic resistance is different from that of SAR in lily. Thus, our approach facilitates the identification of expressed sequences and the exploration of underlying mechanism of induced systemic resistance in the non-model system.

**Ophiostoma and Geosmithia spp. associated with western oak bark beetle damage on declining California black oak and coast live oak in southern California**  
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Phytopathology 101:S249

California black oak (Quercus kelloggi) and coast live oak (Quercus agrifolia) have undergone severe decline in San Diego County, California, since 2002. During a survey of San Diego and Riverside County oak stands in 2009–2010, western oak bark beetle (WOBB; *Pseudopityophthorus pubipennis*) galleries were abundant on branches of both species. Some bleeding from the cambium of B. juncea was also associated with WOBB galleries. Fungi regularly recovered on cycloheximide-streptomycin MEA and tetracycline PDA from necrotic WOBB-infested cambium tissues were identified as *Ophiostoma* sp. and *Geosmithia* sp. by ITS4/5 rDNA sequencing. Pathogenicity of both fungi is being tested by inoculations in field trees to determine if these fungi could play a role in the decline and mortality of oak species in southern California within or outside the goldspotted oak borer (*GSOB, Agrilus coxalis*) zone of infestation.

**Another step closer to implementing inoculum detection as a method to time initiation of fungicide applications for management of grape powdery mildew**  
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(1) Dept. Plant Pathology, Oregon State University, Corvallis, OR; (2) Dept. Plant Pathology, Washington State University, Prosser, WA; (3) USDA-ARS Hort. Crops Res. Lab, Corvallis, OR  
Phytopathology 101:S249

Inoculum detection for timing fungicide applications against grape powdery mildew has been shown to work using qualitative and quantitative PCR approaches. However, these approaches require expensive equipment, specialized skills, and labor costs that impede implementation by viticulturist. Loop mediated isothermal PCR (LAMP) is a robust method for the detection of DNA that can be performed with minimal equipment and skill. A set of LAMP primers were designed against the ITS2 segment of the ribosomal DNA region of *Erysiphe necator* that are specific and can detect less than one spore or less than 5 copies of target DNA in a purified plasmid. Spores were trapped from vineyard air using by continuously running an impaction trap with 40 × 1.5 mm stainless steel rods coated with vacuum grease and replacing sample rods every 3 to 4 days. DNA extraction was accomplished by placing rods in 100 µl of TE buffer, centrifuging for 1 min, boiling for 5 min, vortexing for 10 sec and then placing 5 µl DNA extract in PCR tube with mastermix. The PCR tube was then placed at 65°C for 45 min followed by 80°C for 5 min. Positive detection was determined by the formation of white precipitate. Grower implementation was tested by placing 3 traps at each vineyard with one processed by the grower using LAMP and the other processed in the LAB for LAMP and quantitative PCR. Grower implementation (4) is being tested by placing 3 traps at each vineyard with one processed by the grower using LAMP and the other processed in the lab for LAMP and quantitative PCR. The results of the grower implementation will be presented.

**Comparison of biofungicides and boscalid for management of Sclerotinia drop of lettuce**  
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Phytopathology 101:S249

In Arizona, Sclerotinia drop of lettuce is caused by *Sclerotinia minor* and *S. sclerotiorum*. A field trial was conducted during the 2009 growing season to compare the level of disease control achieved with several different biofungicides and the conventional fungicide boscalid (Endura). Lettuce was seeded on raised beds in double rows 30 cm apart. At seeding, approximately 2,100 sclerotia of *S. minor* or 800 sclerotia of *S. sclerotiorum* produced in the laboratory were distributed on the surface of each 7.6-m-long plot between the rows of lettuce, then incorporated into the top 5 cm of soil. Products were applied to the bed surface at seeding, before initiation of sprinkler irrigation to germinate seed, and one or more times after lettuce was thinned. Compared to nontreated plots, the mean number of diseased plants at crop maturity in plots containing *S. minor* was reduced 72% by Endura, 70% by Contans, 50% by Humega, 33% by Silmatrix and SoilGard, 27% by Actinovate and 15% by Tenet. In plots infected with *S. sclerotiorum*, the number of diseased plants was reduced 96% by Contans, 76% by Endura, 48% by Humega, 46% by Actinovate, 32% by SoilGard, 28% by Silmatrix and 26% by Tenet.

**Resident biology restricts proliferation of Macrophomina phaseolina in brassicaceae seed meal amended soil**  
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(1) USDA-ARS, Wenatchee, WA, U.S.A.  
Phytopathology 101:S249

* M. phaseolina is a pathogen of emerging importance in strawberry production systems. Studies were conducted to assess the efficacy of brassicaceae seed meal amendments for control of this pathogen and to determine the relative importance of soil biology and chemistry in any observed disease suppression. Seed meals were sourced from *Brassica napus* (canola), *Sinapis alba* (white mustard) and *Brassica juncea* (oriental mustard). When inoculated with *M. phaseolina* all seed meal amended soils limited persistence of the pathogen relative to the non-treated control. This was observed irrespective of whether a biologically active chemistry (e.g. allyl isothiocyanate by *B. juncea*) was produced in response to the seed meal amendment. When assays were conducted in pasteurized soils infested with the pathogen, all seed meal amendments failed to suppress *M. phaseolina*. Seed meals also effectively suppressed disease development when strawberry was planted in a soil naturally infested with *M. phaseolina*. However, disease suppression was temperature sensitive, and exhibited failure as soil temperature was elevated above 32°C. Exposure to AITC emitted from *B. juncea* amended soils for up to 8 h had a fungistatic but not fungicidal effect on *M. phaseolina*. These findings indicate that the resident soil biology is the dominant mechanism contributing to suppression of *M. phaseolina* in response to brassicaceae seed meal amendments.

**Particle size affects Brassica juncea seed meal-induced pathogen suppression of Rhizoctonia solani AG-5**  
M. MAZZOLA (1)  
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Phytopathology 101:S249

* R. solani AG-5 is a component of the pathogen complex that incites apple replant disease, and is suppressed via multiple mechanisms in response to *B. juncea* seed meal (SM) amendment. Allyl isothiocyanate (AITC) functions in suppression of this pathogen during the initial 24 h period post-seed meal amendment, but thereafter soil biology and specifically resident *Streptomyces* spp. play the dominant functional role in disease suppression. AITC emission was initiated earlier and reached higher maximal concentrations in soils amended with fine particle (<1 mm dia) than coarse particle (2-4 mm dia) SM. This corresponded with the level of disease suppression obtained when *R. solani* AG-5 and SM were introduced concurrently into soils and plant to apple, but not coarse particle SM suppressed apple root infection when applied to soil at a rate of 0.3% (wt/wt). Both fine and coarse particle SM amendment elevated resident *Streptomyces* approximately an order of magnitude by eight weeks post-application. When soil was infested with *R. solani* AG-5 subsequent to this eight week incubation period and planted to apple, both SM types effectively suppressed Rhizoctonia root rot. These findings demonstrate that at the rates utilized particle size will affect the efficacy of chemistry-based, but not biologically-based, suppression of *R. solani* in response to *B. juncea* SM soil amendment.
Verticillium dahliae genes putatively involved in microsclerotia development
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Phytopathology 101:S250

Verticillium dahliae is a soil-borne fungus, causal agent of an economically significant vascular wilt disease. V. dahliae produces persistent resting structures, known as microsclerotia, which are the primary source of disease inoculum in the field. Microsclerotium development has been studied at the morphological level, but little is known about the molecular mechanisms that govern this process. Recent gene expression studies and analysis of the V. dahliae genome sequence have revealed a diverse number of genes that may be involved in microsclerotia development. This study focuses on the characterization of several class II hydrophobin genes, and a gene ( provisionally designated VdHyp04) that encodes a hypothetical protein. Bioinformatics analyses revealed signal peptide and cleavage sites in all of the hydrophobin-like proteins as well as in VdHyp04, suggesting that all of these proteins are secreted. A methodology that depends on Agrobacterium tumefaciens-mediated transformation is being used to create knockout mutants for these genes. The system involves the transfer into a wild-type Fertillcium strain of a non-functional copy of the gene of interest, and its subsequent homologous integration into the fungal genome to replace the wild-type locus. Transformants are being screened to identify strains in which the mutant gene has replaced the wild-type gene by homologous recombination. Morphological and molecular analyses of these mutants will be done to determine if these genes are involved or not in the microsclerotia development process, and data from these studies will be presented.

Genetic diversity within a vegetative compatibility group of aflatoxin-producing fungi
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Phytopathology 101:S250

Aflatoxin contamination of maize by Aspergillus flavus occurs frequently in both tropical and subtropical climates. Consumption of contaminated commodities adversely influences the health of both humans and domestic animals. Communities of Aspergillus flavus from maize field soils in the State of Sonora, Mexico were dominated by a single vegetative compatibility group (VCG SON003) in 2006. Communities’ structures of aflatoxin-producing fungi have been studied for decades but similar dominance has not previously been reported. Soil samples were collected in 4 agroecological zones across 300 km at elevations ranging from 6 m to 2100 m. In 2007 and 2008, the presence of VCG SON003 was drastically reduced. SON003 is also present in fungal communities resident in the U.S.A., where it has been reported from several states since 1987. Microsatellite markers were used to assess genetic diversity within SON003 before, during and after its dominance in 2006. 292 SON003 isolates were screened for toxin-producing ability; 99% of isolates produced an average of 5789 μg/kg of aflatoxins. Results suggest explosion and dominance by a single clone of SON003 not detected prior to 2006. Implications of these observations on our understanding of the population biology of Aspergillus flavus will be discussed.

Biofungicides as transplant and soil treatment in the control of Phytophthora blight on chile pepper
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Phytopathology 101:S250

Phytophthora capsici is a serious pathogen of a wide array of vegetable crops worldwide. On chile pepper (Capsicum annuum), P. capsici affects all plant parts, and commonly causes plant wilt as a result of severe damage inflicted to the root system. Management of Phytophthora blight requires a system approach. Control of this disease by use of biorational is an emerging area of research. This study was conducted to determine the effects of transplant and soil treatment with biofungicides on the development of Phytophthora blight on chile pepper. Transplants (6-to-8 leaf stage) of AZ-20, a chile cultivar susceptible to P. capsici, were immersed in 0.1% suspension of two biofungicides: Actinovate and Mycostop Mix and water (control) three days prior to transplanting into 12-cm round plastic pots filled with sterilized Terra-Lite Metro Mix 360. Soil was treated by drenching with the following products: 0.1% Mycostop Mix, 0.1% Actinovate, Ridomil Gold EC (1.16 liter/ha). Three days following soil drenching, treatments were inoculated with an isolate of P. capsici at inoculum concentration of 10,000 zoospores per pot. Plants were monitored regularly, and disease severity was assessed weekly for six weeks. Disease severity was not significantly affected when transplants were treated with Actinovate and Mycostop Mix. Across all transplant treatments, soil treatment with Ridomil Gold EC significantly reduced disease severity over 50% compared to control. An effective soil treatment that reduces soil inoculum potential is essential in the management of P. capsici.

Characterization of VvBsl-1 and VvBsl-2 genes of Vitis vinifera in response to Botrytis cinerea
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Phytopathology 101:S250

Chile is one of the most important grapes-exporting countries in the world. For this reason, grapes quality and pathogens control are always required. One of the most relevant grapevine pathogens is Botrytis cinerea, a necrotrophic fungus that produces the grey mould disease. The molecular mechanisms controlling grey mold disease development are poorly known. The objective of this work is to understand this interaction at the transcriptional level. Using a bioinformatic approach, we identified two gene models (named VvBsl-1 and VvBsl-2) with high identity to one Arabidopsis thaliana gene required for susceptibility to Botrytis cinerea. These genes are transcription factors of the R2R3MYB Family and they are grouped with Arabidopsis genes cLade that response to Botrytis cinerea and ABA. It is known that members of this family are required to regulate important processes of the berry development, such as sugar and anthocyanin biosynthesis and antioxidants production. Both genes are induced during different stages of Cabernet Sauvignon berries development. We analyzed the expression of these genes in Cabernet Sauvignon grapes by Real time PCR. We found that VvBsl-1 and VvBsl-2 are highly induced by infection of Botrytis cinerea at 40 hours post-inoculation when compared to non inoculated leaves. Additionally, we analyzed the expression of these genes in others cultivars such as Kyoho, Fuse and rootstocks, in Botrytis cinerea infected leaf and berries at different fruit development stages, as well as uninfected tissue.

Effects of fungicides on a mycopagous coccinellid may represent integration failure in disease management
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Phytopathology 101:S250

The adults and larvae of halyzine coccinellids (Coleoptera: Coccinellidae: Halyzini) are obligate mycophages on hyphal and conidia of powdery mildew (PM) (Erysiphales) fungi, plant parasites warranting chemical control in many managed systems. These insects have been observed to reduce PM severity through high consumption. Fungicide applications, however, may interfere with this ecological service. Five commercial fungicides were topically applied to the mycopagous coccinellid Psylllophora vigintimaculata in the laboratory to gauge contact toxicity. In order to detect interference in the field, population density of naturally occurring P. vigintimaculata was assessed weekly in a northern California vineyard (Vitis vinifera, cultivar “Chardonnay”) over three years in relation to PM (Erysiphe necator) severity and the presence of various PM-antagonistic fungi. Wettability surface contact between adults in the laboratory, resulting in complete cohort mortality 24 hours after application. Topical applications of a streptomycin fungicide (trifloxystrob) and a demethylation inhibitor fungicide (myclobutanil) also resulted in significant adult mortality. Rapid and complete larval mortality was observed in the laboratory after contact with wettable sulfur and myclobutanil. There was no effect on survival after contact with the PM-antagonistic bacterium Bacillus subtilis. Vineyard density of P. vigintimaculata was reduced in vines receiving applications of sulfur, myclobutanil, and several stobilurins, even when considering the covariate PM severity. The microbial antagonist Streptomycyes lydicus did not significantly affect insect density. This study questions the integration of chemical disease management with naturally occurring mycopagous agents in some agricultural systems.

Influence of thrips control programs on TSWV incidence in Fresno County processing tomatoes
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Phytopathology 101:S250

Tomato spotted wilt virus causes substantial losses in California processing tomatoes. Insecticide programs are used to limit disease spread by killing the thrips vector, primarily Frankliniella occidentalis, but the benefit of these programs has not been documented under San Joaquin Valley conditions. A field study was conducted in 2009 to evaluate affect of soil- and foliar-applied
insecticide programs on incidence of TSWV. On 14 May, processing tomato plants cv. H 8004 were transplanted at a research center. A four replication split block design was used. Main plot treatments a) Platinum on 3 Jun, b) Platinum on 3 Jun followed by Venom on 7 Jul, and c) untreated control were applied through a buried drip irrigation system. Subplot treatments were the foliar applications: a) Radiant, Dinomote EL, Lannate WP, and Radiant applied 14 days before silking, b) Venon applied 14 days after silking, and c) ‘treatment a’ without the last Radiant application and d) untreated control. Twenty-five flowers per plot were collected over time and numbers of thrips were recorded. Plants were carefully inspected for TSWV-symptoms and representative samples were confirmed with TSWV immunostrips. Results show that foliar applications reduced thrips counts, but the direct treatment did not have a significant effect. Percent disease incidence on 2 Sep was similar among the three foliar treatments (23.5, 20.3 and 23.2) and significantly lower than the untreated control (33.2). Although only one season of data is presented, it suggests that foliar applications play a role in a TSWV management program.

Characterization of the powdery mildew fungus occurring on evergreen Rhododendrons in the Pacific Northwest

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Phytopathology 101:S251
Powdery mildew is a disfiguring disease of deciduous and evergreen Rhododendron species. Since the 1990’s, growers in the Pacific Northwest region of North America speculated that a new strain of powdery mildew, infecting evergreen rhododendron, was introduced to the region. In order to test this hypothesis, morphological features of powdery mildew fungi infecting Rhododendron were characterized microscopically and phylogenetic analyses of ITS, 28S, and RPB1 sequences were performed. Specimens were collected from diseased ericaceous plants in botanical gardens and residential gardens in Oregon, Washington, and British Columbia. Anamorphs infecting evergreen rhododendron were morphologically similar to those infecting deciduous azalea, with kinked foot cells and singly-formed conidia. Collections from deciduous Rhododendron could be assigned to Erysiphe albo-atrum based on the basis of morphological features, including chasmothecial appendages. Maximum Parsimony and Maximum Likelihood analyses of sequence data suggested that E. albo-atrum may represent a paraphyletic group. Results were consistent with the hypothesis that powdery mildew of deciduous Rhododendron is caused by a taxon distinct from that infecting evergreen Rhododendron. However, these results suggested that the ITS, 28S and RPB1 sequences used are not sufficient to resolve, with confidence, phylogenetic and taxonomic groups within the deciduous Rhododendron-infesting Erysiphales.

Characterization of ATG8 autophagy gene homologs in Verticillium dahliae and Verticillium albo-atrum

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Phytopathology 101:S251
Both Verticillium dahliae and V. albo-atrum are soil-borne fungal pathogens that cause vascular wilt in many economically important plant species. While V. dahliae produces melanised resting structures called microsclerotia (MCS) that can persist in soil for up to 15 years and pose a significant challenge to disease control, the closely related V. albo-atrum survives by forming dark resting mycelia (DRM). To identify candidate genes responsible for resting structure development, cDNA libraries were constructed from cells grown in two environments; i) a simulated xylem fluid medium where the fungus exhibits dimorphic growth, and ii) conditions that favour near-synchronous microsclerotia development. Sequences highly similar to genes encoding the well-characterized yeast macroautophagy marker ATG8 were identified in both cDNA collections, indicating a role for autophagy in Verticillium development. In other filamentous fungi, autophagy has been shown to be required for nutrient recycling during starvation, and also to be involved in cellular differentiation and accompanying developmental processes such as germination, sporulation, and infection structure formation. We are characterizing the ATG8 genes of V. dahliae (Vd) and V. albo-atrum (Va). ATG8 gene knockout mutants have been generated in both species, and comparative morphological studies between wild-type and knockouts have shown that VdATG8 is involved in condensation, dimorphic growth, and microsclerotia formation in V. dahliae. Morphological defects were observed in VaATG8 mutants. Intriguingly, stressing the fungus by increasing the temperature, results in restoration of the wild-type phenotype in the vdatg8 strains. Recent data from other morphological and microscopic analyses of the mutants and complemented strains will be described.

Relationship among kernel drydown rates, environmental factors and resistance to giberella ear rot, fusarium kernel rot and common smut of corn

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Phytopathology 101:S251
Giberella ear rot (Fusarium graminearum), fusarium kernel rot (F. verticillioides) and common smut (Ustilago maydis) are three ear diseases of corn which frequently occur in Canada and many other countries. These diseases reduce yield, quality, and the two fusarium pathogens may contaminate the grain with mycotoxins. Many studies have indicated that there is a short window of time in which a corn ear can be infected and that this window is greatly influenced by the environment. We hypothesized that genotypes with faster kernel dry down rates would be less infected as they would ‘escape’ colonization. In 2008 and 2009, six corn inbreds of varying resistance to each disease and eight F1 hybrids between some of these inbreds were used in a study to investigate the relationship between kernel drydown rate (KDR), susceptibility to the three ear diseases and environmental factors (corn heat units, total rainfall and global solar radiation) from silking to eight weeks after mid-silk. Six inoculation treatments were used: 1) F. graminearum, kernel inoculation; 2) F. graminearum, silk channel inoculation; 3) F. verticillioides, kernel inoculation; 4) F. verticillioides, silk channel inoculation; 5) U. maydis; and 6) a sterile water control. Ear moisture was measured at five and eight weeks after pollination, using an electronic probe and used to calculate KDR. Significant (P < 0.01) genotype and treatment effects were found for KDR and disease severity. Statistically significant correlations were found with KDR for all three diseases and all inoculation techniques with the highest correlation for KDR and kernel inoculation with F. verticillioides. Correlations were also found between symptoms and some of the environmental factors investigated. It was concluded that KDR does play a role in the severity of disease symptoms for these three diseases.

Characterization and overexpression of L-amino acid oxidase from Trichoderma harzianum ETS-323

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Phytopathology 101:S251
In our previous studies, a putative L-amino acid oxidase (LAAO) that was secreted by Trichoderma harzianum ETS 323 has been suggested to involve in its antagonism with Rhizoctonia solani. However, research which has empirically investigated the biochemical properties of L-amino acid oxidase from T. harzianum ETS 323 is scant. Therefore, the aim of this study attempts to explore the essential feature of L-amino acid oxidase of T. harzianum ETS 323 including its protein sequence, catalytic activity and specificity for amino acid substrates. Currently, the T. harzianum ETS-323 LAAO (Th-LAAO) gene was overexpressed and purified from Escherichia coli. To our knowledge, this is the first overexpression of LAAO from Trichoderma. The amino acid sequence alignment analysis using the ClustalW revealed that Th-LAAO exhibited a highest identity (98%) with the T. harzianum mRNA for LAAO. The purified protein showed a molecular mass of 52 kDa in sodium dodecyl sulfate-polyacylamide gel electrophoresis (SDS-PAGE) and catalyzed H2O2 formation from the L-phenylalanine and L-histidine, thus confirming its LAAO activity, where the specific constant (kcat/Km) value of each was 0.11 and 0.06, respectively. To conclude, this study may be of importance in providing researchers with a better understanding of LAAO’s biological function from Trichoderma.

Species of Neofabraea responsible for anthracnose canker of apple trees in western Washington State

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Phytopathology 101:S251
To determine species of Neofabraea responsible for apple anthracnose canker in eastern Washington, apple tree crown tissue samples with anthracnose canker were collected in November 2007 from seven apple orchards in the Bellingham and Mt. Vernon areas, WA. A total of 146 isolates of putative Neofabraea spp. were obtained from the disease samples. All isolates were single-sporule cultured. Total genomic DNA was extracted from cultures of these isolates. Amplification of DNA was performed in a multiplex PCR using four species-specific primers to identify isolates to species. Of the 146 isolates identified, 142 were N. malicorticis, 2 were N. alba, and 2 were Cryptosporiopsis kienholzi (the anamorph of a Neofabraea sp.). No N.
perennans was found among the isolates tested. To test pathogenicity of these species on apple, 2-year-old twigs of Jonagold apple were wounded and inoculated with one representative isolate each of *N. malicorticis*, *N. alba* and *C. kienholzii*. Twig inoculations were conducted twice (late October and late November 2008) in the orchard. Sizes of canker were measured 2 or 3 months after inoculation. All three species were able to cause cankers on inoculated twigs, and the same fungi were re-isolated from diseased twigs. The results indicate that *N. malicorticis* is the major cause of anthracnose canker of apple trees in western WA and that *N. alba* and *C. kienholzii* also are able to cause cankers on apple trees.

**Pathogenic races of Exserohilum turcicum on corn in Ontario and Québec**

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Phytopathology 101:S252

Northern corn leaf blight (*Exserohilum turcicum* = *Helminthosporium Turcicum*) incidence in Canada has increased from 2002 to 2009. Incidence in surveyed corn fields reached 65.5% in Ontario in 2009 and 72.7% in Québec in 2008. Evidence was also found indicating that ‘resistant’ genotypes were expressing susceptible lesions. To identify the pathogenic races of *E. turcicum* that exist in these two provinces, 157 samples of diseased leaf tissue from 21 counties in Ontario and 39 samples from 16 counties in Québec were collected in 2005 and 2006, respectively. A single lesion from each sample was used for race identification. The lesion was divided into two halves, one was allowed to sporulate and used to inoculate greenhouse grown corn plants in the winter of 2006–2007; the other half was used to inoculate field grown corn plants in 2007. Six corn inbred lines, A619, A619Ht1, A619Ht2, A619Ht3, A632HtN, H102, were inoculated; these inbreds have the 0, Ht1, Ht2, Ht3, HtN, and Htm major resistant genes, respectively. In the greenhouse all of the 196 samples expressed on host either a resistant (R) lesion with a yellow margin which sometimes developed a gray centre or an elliptical, gray coloured susceptible (S) lesion; in the field, 195 sampled lesions expressed these symptoms. In the greenhouse, the ratio of R:S was 0:196, 161:35, 115:81, 90:106, 181:15 and 126:70 for the 0, Ht1, Ht2, Ht3, HtN, Htm hosts respectively; similarly the ratios in the field were 0:195, 23:172, 108:87, 94:101, 108:87 and 183:12, respectively. Ht1 did not show resistance in the field as it did in the greenhouse. We identified 25 pathogenic races in the greenhouse, five with the higher frequency and 21 races were identified in the field, seven of which were higher frequency. Thus the expressions of each resistant gene were very complicated. Further identifications are required.
2010 Caribbean Division Meeting Abstracts

Abstracts presented at the APS Caribbean Division meeting in Managua, Nicaragua, August 24–27, 2010. The abstracts are arranged alphabetically, by first author’s name.

Early detection of Phytophthora palmivora in oil palm, using real-time polymerase chain reaction (QPCR) and molecular beacon probes
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Phytopathology 101:S253

Bud rot is considered the most limiting disease of oil palm in South America because it causes direct production losses on thousands of hectares dedicated to the crop. A Phytophthora sp. was isolated from commercial plantations cultivated in Ecuador, Brazil, and Colombia showing typical symptoms of bud rot. Pathogenicity was evaluated in 6- and 24-month-old palms under controlled conditions in the greenhouse and in leaf fragments placed in humidity chambers. Isolates of this Phytophthora sp. were found to be pathogenic on both 6 and 24-month-old palms, and Phytophthora sp. was re-isolated from infected tissue, therefore fulfilling Koch’s postulates. Through PCR, the causal agent was identified using A2/I2 primers specific to the genus Phytophthora, which amplified a 788-bp fragment located in the internal transcribed spacer (ITS) region. The PCR product was digested with the restriction enzymes MspI, RsaI, and TaqI, generating fragments that corresponded to P. palmivora. With the sequences obtained, molecular beacon probes and primers specific to the species were designed. This new method permitted timely, sensitive, and specific diagnoses of the pathogen in asymptomatic tissue, therefore fulfilling Koch’s postulates. Thus, studies on propagation distribution in soil and pathogen biology were made possible. As a result of this study, reliable decision-making tools for the integrated management of the disease could be developed to manage this disease.

New observations on the role of Lincus sp., in the transmission of sudden wilt (Marchitez sorpresiva) in oil palm
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Phytopathology 101:S253

Sudden wilt (Marchitez sorpresiva) of oil palm has been associated with a protozoon of the genus Phytomonas (Trypanosomatidae), identified as P. staheli. The first symptoms of the disease include loss of fruit luster, followed by rotting of fruits and cessation of flowering. This is followed by foliar browning and desiccation beginning at the tips of the lower leaflets and from the external end of the leaves, and advances upward into the crown in less than two months after first symptoms. There is also dead of the roots. Only mature, bearing palms develop symptoms in the field. There are some studies that indicate that the pathogen is transmitted by the Pentatomidae Lincus sp., but they do not indicate, clearly enough, the relationships between the vector, the pathogen and the palm. In this study, 15 years old palms as well as 80 year old nursery palms were inoculated with insects collected from diseased palms, 20 per palm in the adult ones and five in the young ones. As control there were used insects collect in an area free of this disease. They were kept on the experimental plants for 45 days. To recognize the presence of the pathogen associated with MS, the inoculated palms were evaluated 60, 100 and 300 days after the inoculation: In none of the inoculated palms it has been possible to observe symptoms of this disease or the presence of flagellated in the roots. It is necessary to get better information about the real role of Lincus sp., as vector of P. staheli in oil palm and if there are other Pentatomidae, Lygaeidae, or Coreidae insects, involved in this process. It is required to know more about the relationship between the vector and the pathogen in order to improve the control measures for this disease.

Phytopathogenic fungi of immature mango (Mangifera indica L.) fruits
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Phytopathology 101:S253

Colonization of immature mango fruits (mean = 1.6 cm) by phytopathogenic fungi is a key aspect of the production cycle. Their identification and description can lead to better disease control and management. Early ripeness, necrosis and soft rot are symptoms frequently observed in immature mango fruits in the field. Pathogenicity tests were conducted to determine the relative importance of phytopathogenic fungi isolated from symptomatic tissues of immature fruits. Healthy immature fruits were collected from mango cultivars Haden, Irwin, Parvin and Keitt at the Agricultural Experimental Station in Juana Diaz, Puerto Rico. The fruits were transported on ice to the laboratory, superficially disinfested and inoculated with a mycelial disc of axenic cultures of each fungal isolate. The following fungi were tested: Colletotrichum spp., Fusarium spp., Guignardia mangiferae, Phomopsis spp. and Botryosphaeria spp. Inoculation treatments consisted of fruits with and without wound including a non-inoculated control under laboratory conditions. Assays were examined in triplicates. After incubation at 25°C for 7 days, fruits were examined and fungi reisolated in acidified PDA. Ten fungal isolates caused necrosis and soft rots similar to those observed in the field with the exception of Botryosphaeria sp. (C isolate). Botryosphaeria spp. (D isolate) caused damage only in fruits with wounds suggesting the opportunistic strategy of this species. A better understanding of the diversity of phytopathogenic fungi colonizing mango fruits at early stages could lead to better prediction tools to diminish eventual damage of mature fruits.

Identification of Golovinomyces sp. in Acalypha wilkesiana using Scanning Electron Microscope and other classification criteria
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Phytopathology 101:S253

Powdery mildews are obligate parasites of the Order Erysiphales, Phylum Ascomycota. These fungal pathogens are of great economic importance causing severe losses in the ornamental industry in Puerto Rico. Powdery mildews infect almost all ornamentals in greenhouses and gardens and appear as a white to grayish growth on leaves and young shoots. Acalypha wilkesiana (Ima), from the Euphorbiaceae family, is a perennial shrub widely distributed in the Island and affected by powdery mildew. Observations using light and scanning electron microscope revealed ellipsoid, hyaline conidia borne single and terminally on the conidiogenous cell distinctive of Golovinomyces sp. Other morphological criteria examined were nipple shaped appressoria, absence of fibrosin bodies and amphiogenous mycelium of the anamorphic stage. Measurements taken from primary conidia from infected tissue were on average 28.62 µm (length) × 14.01 µm (width). In addition, Ampelomyces quisqualis, a hyperparasite of powdery mildews, was
identified in acalifa samples by sequence analysis of the ITS region of the ribosomal DNA.

**Rhychnophorus palmarum and Strategos aloeus management in oil palm plants affected by bud rot disease in Colombia**

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Phytopathology 101:S254

Bud rot disease in oil palm (Elaeis guineensis Jacq), is the most severe disease in Colombian and also in all tropical America where oil palm is cultivated. Specifically, in Colombian, bud rot disease has destroyed thousands of hectares. The situation is more severe because the presence of the insect Rhychnophorus palmarum (Coleoptera: Curculionidae) that is attracted by the plants affected by this disease. On these plants R. palmarum female lays its eggs and emerged larvae feed on the damaged plants. It avoids the recovery of plants affected by bud rot that normally dies by the cumulative effect of both negative factors. Another important issue is that R. palmarum acts as primary vector for red ring nematode, Bursaphelenchus cocophilus. Diseases occurrence, red ring and bud rot on oil palm plants had caused eradication of thousands of hectares, that result in the amount of plants stalls, where reproduces. At the same time Strategos aloeus (Coleoptera: Scaranidae), causes direct damage in the bulb in plants with less than four years in the field. It has been observed that there is a relation between S. aloeus and R. palmarum, where R. palmarum uses the galleries done by S. aloeus and also damages young plants. In this work we present activities that the Colombian Oil Palm Research Centre, Cenipalma- is developing, in the management and control of both insects. Among these activities are the developing of a integrated pest management based in R. palmarum trap, access of biological control for both coleopteran and study of new molecules that will permit specific pest control.

**Identification of Oidium neoly copersici, a powdery mildew of tomato, using scanning electron microscopy (SEM) and DNA analysis**

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Phytopathology 101:S254

Tomato production in Puerto Rico is valued at $11 million per year. Powdery mildews (Ascomycetes, Order Erysiphales) are an important limiting factor in tomato production. Commonly, the foliage is covered with a profuse white-to-grayish growth, which often results in reduced yield. Recent research has established the existence of two powdery mildews species affecting tomato: Oidium lycopersici, restricted to Australia and O. neoly copersici, a newly described species of worldwide distribution. The objective of this research was to identify the Erysipheles attacking tomato in Puerto Rico. Tomato leaf samples with powdery mildew symptoms were field collected. Species determination was performed using both, light and scanning electron microscopy. In addition, PCR was used to amplify DNA’s ITS region, followed by sequencing. Criteria for species determination were conidial production and germination, shape of appresoria, presence or absence of fibrosin bodies, and fimbriate patterns of the conidial wall. Using these criteria, we can confirm the presence of the newly described O. neoly copersici infecting tomatoes in Puerto Rico.

**New developments in the study of the causal agent of oil palm bud rot disease in Colombia**


Phytopathology 101:S254

Bud rot is being considered the most serious disease of oil palm not only in Colombia but also in neighbor countries. Even though the disease has been studied for more than 40 years in Central and South America, the causal agent remained unknown for many years. As the result of the work done by Cenipalma, it was possible to identify Phytophthora palmivora as the causal agent for this disease. The pathogenicity tests are an important tool in the evaluation of materials for its tolerance to the disease. The researchers at Cenipalma have been working in the development of a reliable test for this purpose. One of them has been the inoculation of very young leaves on a leaf cutting in *in vitro* conditions. The other one has been the inoculation of nursery palms. This work describe the procedures and the results obtained in the pathogenicity tests, and complements the previous ones that made it possible to identify *P. palmivora* as the causal agent of bud rot in oil palm. In the *in vitro* tests it is possible to see the rapid colonization of the tissue by the pathogen and in the nursery palms it has been possible to obtain up to 100% infection. In both cases it has been possible to see the advance of the mycelium toward healthy tissue and the development of sporangia as well as the release of more zoospores for further infection. Having a reliable procedure for pathogenicity tests is a very interesting advance in the study of this disease, the confirmation of its causal agent, and the development of very useful tools for the new studies, that are required for the evaluation of different isolates and for the breeding activities oriented toward the identification of sources of partial resistance to this disease.

**Early diagnosis, elimination of affected tissue and protection of surrounding palms: The key for control of Phytophthora palmivora, the causal agent of bud rot in oil palm in Colombia**


Phytopathology 101:S254

Bud rot, produced by *Phytophthora palmivora*, is the most important disease of oil palm in all the production areas of Colombia. With the recognition of the first symptoms as necrotic lesions on the sides of the young spear leaves (youngest, unexpanded leaf), it has been possible to teach palm workers how to do early identification of infected palms and to proceed with a treatment for the removal of affected tissue. When a diseased palm is identified, there is an inspection to decide the best area to access the upper part of it and with a sharp chisel, previously prepared, it is eliminated the least number of mature leaves necessary to reach the affected tissue. This is removed below the area of advance of the lesions produced by *P. palmivora*. All the tools used and the boots of the workers are disinfected with a solution of sodium hypochlorite. The exposed tissue is protected with a paste prepared with a fungicide, a bactericide and an insecticide. The surrounding palms are treated with a cocktail of fungicide, bactericide and insecticide to protect them during several weeks, depending of the inoculum pressure in the area and the main environmental conditions. Plants in advanced stages of the disease are eradicated. Using this procedure it has been possible to reduce the impact of the disease not only under experimental conditions but also in commercial plots in different growing areas. Once it was known the disease agent, it was easier to develop the control measures that must be implemented as soon as possible, to avoid high incidences of this disease. This procedure is being implemented by many farmers in different affected areas, and whenever the procedure has been done correctly, and especially when it has been implemented with low incidences, the results have been very promising.

**Preliminary observations on basal stem rot in oil palm in Colombia**

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Basal stem rot associated (BSR) with *Ganoderma spp.*, and particularly *G. boninense*, has been considered one of the most important diseases in oil palm. In Malaysia and Indonesia it is responsible of big losses due to yield reduction of more than 80%. In Colombia and also in all tropical America where oil palm is cultivated, basal stem rot is an important limiting factor. The surrounding palms are treated with a cocktail of fungicide, bactericide and insecticide to protect them during several weeks, depending of the inoculum pressure in the area and the main environmental conditions. Plants in advanced stages of the disease are eradicated. Using this procedure it has been possible to reduce the impact of the disease not only under experimental conditions but also in commercial plots in different growing areas. Once it was known the disease agent, it was easier to develop the control measures that must be implemented as soon as possible, to avoid high incidences of this disease. This procedure is being implemented by many farmers in different affected areas, and whenever the procedure has been done correctly, and especially when it has been implemented with low incidences, the results have been very promising.
Oil palm bud rot disease (BR) is the main limiting factor for the production of the crop in Colombia. The disease has been rapidly growing in the last few years with devastating effects in some of the producing zones in the country. BR is found in the different oil palm development stages from nursery stage to established palms in the field. For many years, several microorganisms have been associated with the disease, however *Phytophthora palmivora* Bult, is now recognized as the causing agent of the initial lesions that finally lead to the rotting process typical of BR. Nevertheless, it is clear that the symptoms associated with the disease involve other microorganisms besides *P. palmivora*. In order to identify fungi and oomycetes associated with the rotting process, isolates were obtained from BR affected palms collected in three producing zones in Colombia. Microorganisms were purified and then identified based on rDNA ITS region sequencing using ITS1 and ITS4 primers. ITS regions from 117 isolates were sequenced. Based on the sequencing results 35 species were identified, including 6 oomycete species (*Phytophthora* spp. and *Pythium* spp.), 16 ascomycetes and 13 basidiomycetes. The results showed the predominance of distinct associated species of *Fusarium* (F. solani, F. oxysporum, F. equiseti, Gibberella zeae and G. moniliformis), representing 38% of the identified isolates. Thielaviopsis paradoxa (7.7% predominance) and *P. palmivora* (4.3% predominance) were also present. New species of genus never found before associated with BR disease were also found, including some well recognized oil palm endophytes. Based on the results we are developing molecular tools for microorganism identification and BR diagnosis.

Applications of PCR-based techniques in the diagnostics of tropical plant pathogens

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Phytopathology 101:S255

Molecular techniques based on the amplification of nucleic acids, such as Polymerase Chain Reaction (PCR), are routinely used in the diagnostic determinations of plant pathogens worldwide. Their advantage consists on their specificity, sensitivity, strength, consistency and speed, in contrast with traditional techniques. Application of PCR-based methods to plant pathology problems in tropical agrosystems has provided evidence that complements traditional techniques in the identification of important pathogens. Our experience using PCR-based techniques has allowed us to identify *Colletotrichum musae* in mango leaves, *Fusarium* sp. in *Garcinia* in Florida and Puerto Rico. Recently, we have used analysis of rDNA ITS region to identify five fungal species belonging to the Botryosphaeriaceae occurring in tropical fruits inflorescences. In addition, the use of species-specific primers complements the identification of pathogenic vs. non-pathogenic *Guignardia* spp. We have identified *G. mangiferae* an endophyte commonly occurring in rambutan and mango fruits in Puerto Rico from *G. citricarpa*, an important pathogen of quarantine significance in citrus causing black spot occurring in other countries. Unfortunately, DNA sequence data are frequently not available to support accurate diagnoses of many tropical fungal pathogens species. Often, many species are new to science, or sequence data is not available, even for most described species. For example, *Lasmenia* spp., a pathogen of rambutan, we still have to morphologically characterize the pathogenic character and work on obtaining molecular data to submit to the GenBank. We expect that increased availability of reliable DNA sequence data for tropical fungal pathogens will make faster and more accurate identification possible.

Examining soilborne pathogens as causal agents of the decline of *Torrey saxifolia*

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*Torrey saxifolia* Arn. is an evergreen conifer endemic to the slopes of the Apalachicola River in Florida and Georgia. Surveys suggested that *T. saxifolia* has lost at least 98.5% of its total population size since the early 1900s, causing that species be federally listed as endangered. Given the lack of seed production in the wild, and potentially a decline due to a fungal disease, all populations are viability models predict extinction. To determine the potential importance of soil-borne pathogens associated with *T. saxifolia*, we conducted a systematic survey of soil-borne fungi. Twenty four individuals, showing different degrees of decline, were sampled at two different sites: Torrey State Park (TSP), Florida, and US Corps of Engineers (Corps) land tract in Decatur, Georgia. The TSP trees were smaller (89 cm h; 5 cm diam.) than the Corps trees (105 cm h; 10 cm diam.). In addition, root rottedness and leaf blight symptoms were observed in 46% of trees examined. A diverse fungal community was associated with declining trees. Twenty fungal genera were identified belonging to the Oomycetes, Zygomyctes, Ascomycetes, Basidiomycetes, and anamorphic fungi. Anamorphic fungi, Oomycetes and Zygomyctes were the dominant groups. The majority of the isolates were obtained from root tissue. Seventy five percent of isolates were obtained from the Corps trees. Soil inhabiting fungi and rhizosphere could play an important role in the decline of *T. saxifolia* and pathogenicity tests are crucial to finally demonstrate their role.

Control measures for lethal wilt in oil palm in Colombia

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Lethal wilt is one of the emerging diseases of oil palm in the East and Central growing zones in Colombia. Its causal agent has not been identified, neither the way it is being disseminated in the field. There are some reports indicating the association with a phytoplasma, but it has not been proved with certainty. A similar disease was reported in the 1970s in Northeast Colombia. The first symptoms of the disease are loss of fruit luster, rotting of fruits and roots, followed by foliar browning and drying beginning in the lowest fronds, at the tips of the leaflets and from the external end of the leaves, and advances upward into the crown in less than two months after first symptoms. Only mature, palms develop symptoms in the field. In order to develop control measures and under the presumption that there is an insect involved in transmission of the disease and that the grasses present in the oil palm fields are competing with the palm for water and nutrients and also are substrates for insect breeding. It was implemented a management program with insect control, weed control or both, to identified the best control measures. After more than 30 months of observations it was spread of the disease with the control of the vector involved in this disease transmission. The control measures, similar to the ones implemented for a similar disease in the 1970s in Northeast Colombia, appear to be the procedure to follow for the management of ML. With this information there are new possibilities to continue the studies to identify the vector as well as its causal agent.

Witches'-broom in Guacamo (*Guazuma ulmifolia*, Sterculiaceae), a disease caused by a phytoplasma from the 16S rXV group in Costa Rica

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Guacamo (*Guazuma ulmifolia*) trees showing witches'-broom (GBW), reduced leaf size, short internodes, general stunting, and no flower and fruit production were observed on side roads in North, Pacific and Central Western areas of Costa Rica. To determine the occurrence of phytoplasma infection in symptomatic trees, analyses based on transmission electron microscopy (TEM), nested-PCR (primers P1/16S-DR followed by R16F2a/R16R2, RFLP (Alu, HpaII, Tru1I and Tsp509I) and sequencing were performed. Phytoplasmas were observed in the sieve cells of symptomatic trees but not in healthy ones by TEM. The infection was confirmed by nested-PCR, generating amplicons (about 1.2 kb) from all DNA samples from symptomatic trees. The RFLP analysis showed identical patterns among GWB samples and indicated that this phytoplasma belonged to hibiscus witches'-broom group (16S rXV). The 16S rDNA sequence (1400 nt) obtained from two GWB phytoplasma strains, shared 98.8% similarity with * Candidatus Phytoplasma brilliense* (AF147708). The virtual RFLP pattern showed 95% similarity with subgroup 16S rXV-A (AF147708), suggesting that this strain may represent a new subgroup within 16S rXV group. This is the first report of a phytoplasma infecting the neotropical tree species *G. ulmifolia*, and the natural occurrence of a phytoplasma strain closely related to *C. P. brilliense* in Costa Rica.

Interactions between thrips and plant pathogenic fungi in mango inflorescences

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Despite the relative worldwide economic importance of mango (*Mangifera indica* L.), particularly in Puerto Rico, few studies have been conducted on fungal pathogens or on insect populations associated with inflorescences. Thrips (Order Thysanoptera) might cause damage by ovipositing in the panicle, feeding on flower tissues, thus destroying cells and causing open wounds, through which plant pathogenic fungi can enter. Several fungi have been associated with necrotic tissues in mango inflorescences. Our objective
was to evaluate several alternative infestation methods that involve presence or absence of mango flower thrips and 12 different fungal species. Cultivar Irwin was used as it is commonly found in Puerto Rico. Fungal species were: *Alternaria alternata, A. infectoria, Albonectria rigidiascula, Botryosphaeria ribis, B. rhodina, B. dothidea, B. parva, Colletotrichum gloeosporioides, Fusarium moniliforme, Neofusicoccum mangiferae, Phoma sorghina, and Phomopsis longicolla*. Disease severity for each fungal species was evaluated in inflorescences on the following treatments: 1) No thrips and fungal inoculation with artificial wound, 2) no thrips and fungal inoculation without artificial wounds, 3) infestation of inflorescences with thrips, and 4) fungi and thrips in the same inflorescence. Healthy inflorescences were used as controls. Treatments were evaluated at five and eight days after inoculation. The highest disease severity was observed in inflorescences inoculated with fungi with artificial wounds and inoculation of fungi with thrips infestation. Inflorescences treated with thrips and *B. ribis, B. dothidea, B. rhodina, N. mangiferae, P. sorghina* and *P. longicolla* caused up to 100% disease severity. Management strategies in the field should emphasize thrips and disease control to reduced flower and eventually mango fruit damage.
Major plant-parasitic nematodes in the northeast region and challenges they pose to agricultural plant managers

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Large and diverse nematode communities occur in most agricultural soils and play a significant role in soil ecological processes. Soil inhabiting nematodes are generally characterized into functional trophic groups, which include the herbivores (plant parasites), fungivores, bacterivores, omnivores and predators. Relative abundance of these groups has been used as an indicator of soil health. However, several widely distributed genera of plant-parasitic nematodes have been documented to directly and/or indirectly cause significant agronomic crop losses throughout the northeast. At large population densities, plant-parasitic nematodes can directly reduce crop growth and marketable yield. In addition, several are also known to predispose plants and/or interact with other pathogens, pests, and soil organisms to cause plant diseases of complex etiology. Root-knot (primarily Meloidogyne hapla), lesion (primarily Pratylenchus penetrans), dagger (Xiphinema americanum and X. revise), cyst (PCN, Heterodera rostochiensis; SBCN, H. schachtii; CCN, H. trifoli; TCN, H. tabacum), bulb and stem (Ditylenchus dipsaci), and foliar (Aphelenchoides spp.) nematodes are the primary nematode pathogens that require effective management in the northeast. Although management practices continue to emphasize the use of chemical nematicides, significant progress has been made on the use of alternative management options including bio-fumigant cover crops, crop rotations, resistant crop varieties, antagonistic organisms and bio-based IPM strategies. Important diseases caused by plant-parasitic nematodes in the northeast and their management options will be illustrated and discussed during the presentation.

Effect of silicon amendment on the enhancement of soybean resistance to Phakopsora pachyrizi

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Soybean rust caused by Phakopsora pachyrizi is a major threat to soybean production throughout the world. Silicon (Si) could represent an alternative solution to repeated fungicide applications because of its reported prophylactic role against many plant pathogens and in particular biotrophic fungi. However, little is known about the potential effects of Si on soybean because the plant’s ability to absorb Si is poorly defined. Our objectives were 1) to evaluate and quantify the deposition of Si in shoots of soybean plants (cv. Williams 82) fed with various Si concentrations, 2) to determine if there exists a differential ability to accumulate Si among soybean cultivars of various origins and 3) to evaluate if the absorption and subsequent accumulation of Si could enhance the resistance of soybean plants to P. pachyrizi. Scanning electron microscopy and X-ray microanalysis mapping were used to determine Si deposition in soybean leaves of plants treated with 0, 0.4 or 1.7 mM Si and rust severity was assessed daily. The experiment with cv. Williams 82 revealed no significant differences in the plant’s Si content regardless of the Si concentration in the solution. This translated into no effect of the treatment on rust incidence. These results support the concept that no benefits are conferred to a plant that can not absorb Si beyond background levels. On the other hand, cv. Hikmok displayed a significantly higher Si concentration in planta than all others when fed with Si. Interestingly, the same cultivar displayed the highest resistance to P. pachyrizi under Si treatment. This resistance appeared to be mediated in part by a hypersensitive response based on the occurrence of lesions typical of this reaction. Our results suggest a potential role for Si as part of an integrated approach to control soybean rust.

Assessment of butternut health throughout New England and New York

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The butternut canker fungus (Siroccus clavigignenti-juglandacearum) (SCJ), believed an exotic pathogen, has had serious biological, ecological, and economic impacts on butternut (Juglans cinerea) throughout eastern North America. Butternut is a rare species in this region but when found is often associated with early European farms and sites with a history of Native American use. Since limited information was available on the health status of butternut, this study assessed incidence, severity, and mortality of 2034 trees in New England (NE) and New York (NY) (2006–2009). SCJ infection levels were about 95% for butternut in northern NE and NY but somewhat lower in Southern NE (MA, CT, & RI) based on observations of the main stem and root flare areas. However, it is very likely that small twig and branch infections occurred high in the crowns and could not be seen. All trees were assigned a crown class (dominant, co-dominant, intermediate, or suppressed) and then given a health class rating ranging from 1 (relatively healthy) to 4 (dead). Dominant and co-dominant trees had the highest health class ratings (1 & 2) and lowest levels of mortality, whereas the reverse was true for intermediate and suppressed trees. Current butternut mortality levels in ME, NH, NY, & VT were 28.1, 27.3, 24.5, & 29.5 percent, respectively. In MA, CT, and RI, mortality levels were 15.7, 5.0, 5.9, & 5.9, respectively. Virtually no nut production was observed during this study, and seedling and sapling regeneration was woefully lacking and when found, it was usually infected with SCJ. Also, it was apparent that SCJ is not the only pathogenic organism associated with declining trees but is probably the most important. Opportunistic leaf, stem, & root diseases were observed but their relative impact on butternut health remains unknown. Funding: USDA FS FHM and Plant Technologies.

Antagonistic activity of two strains of Pseudomonas against Helminthosporium solani, the causal agent of potato silver scurf

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Silver scurf, caused by the fungus Helminthosporium solani Durieu and Mont., is a surface-blemishing disease spoiling the appearance of potato tubers. The disease has emerged as an economically important disease in the last twenty years. This is mainly due to the appearance of H. solani strains resistant to thiabendazole. As a result, many efforts have been put forth to develop effective biocontrol alternatives to thiabendazole application. Recently, two strains (94-19 and E-30) of Pseudomonas, isolated from suppressive soils, showed strong antifungal activity against H. solani. The aim...
of this work was to further investigate the antagonistic interactions between *H. solani* and these bacteria. A bioassay was first designed to monitor the activity of bacterial extracts against *H. solani*. The effect of the medium on the production of antifungal compounds by the bacteria was then evaluated in vitro. Strain 94-19 produced antifungal metabolite(s) against *H. solani* in all liquid media tested while strain E-30 produced antifungal metabolite(s) only when co-cultivated with living fungal cells, particularly *H. solani* cells. Although the antifungal compounds involved in the antagonistic interactions were not determined, the presence in strain 94-19 of genes for the synthesis of pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol suggests that these compounds are involved. These genes were not detected in strain E-30. The results presented in this study open the way to new avenues of investigation towards achieving biological control of potato silver scurf.

*Armillaria* species distribution and site relationships in *Pinus* and *Tsuga*-dominated forests in Massachusetts

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The distribution of *Armillaria* species was investigated across 32 plots at eight sites within four conifer-dominated forest types (eastern hemlock, pitch pine, eastern white pine, and eastern white pine – oak). In total, 320 isolates were collected from 19 host tree species, with 207/320 (65%) isolations coming from the three primary conifers. To date, 280 isolates have been identified to species using a PCR-RFLP protocol that targets variation within the IGS-1 and IGS-2 regions of the rDNA cluster. All six northeastern *Armillaria* species (*A. abietis*, *A. gallica*, *A. gemina*, *A. mellea*, *A. sinapina*, and *A. solidipes*) have been encountered in this study. Overall, *A. solidipes* (sensu *A. ostoyae*) was the most abundant species encountered, making up 161/280 isolates. While this species prefers conifers, it was found on hardwoods 39 times (24%). In contrast, *Armillaria* species typically associated with hardwoods were frequently encountered on conifers. *Armillaria mellea* (sensu stricto) was collected a total of 26 times from eastern white pine and pitch pine, and *A. gallica* was found a total of 27 times on eastern hemlock, pitch pine, and white pine. *Armillaria* species incidence was significantly different by forest type, as pitch pine forests had a higher incidence of *A. solidipes* (70/80; *p* < 0.001), and eastern hemlock forests had a greater incidence of *A. gallica* (32/80; *p* < 0.001), compared to expected values. Incidence of *A. solidipes* was significantly different by soil type, with a greater incidence on excessively drained, sandy soils (*p* = 0.006). Occurrence by crown class was also significant, with a greater number of infected pitch pine (*p* = 0.001) and white pine (*p* < 0.001) occupying the intermediate and suppressed crown classes. The results illustrate how assumptions of *Armillaria* species incidence by host can be erroneous in eastern forests where hardwoods and conifers are mixed.

Effect of microbial communities in recycled irrigation water on the development of three *Pythium* species

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A concern in commercial greenhouse production is the harboring of *Pythium* species in recycled irrigation water and spreading to susceptible crops. *Pythium* species are among the most damaging pathogens in horticulture causing damping-off, root rot, and stem rot. Despite frequent attempts to recover *Pythium* from recycled water, success in isolation is sporadic. The main objective is to determine if microbial communities recovered from recycled irrigation water from commercial greenhouses have a deleterious effect on the growth, reproduction, and survival of three pathogenic species, *Pythium aphanidermatum*, *P. irregulare*, and *P. cryptoirregulare*. The identification of bacteria exhibiting in vitro inhibition in the development of *Pythium* may be useful in development that can be implemented as alternative strategy to control disease caused by *Pythium* because such organisms could be grown and added to irrigation systems. Microscopic observations of the interaction between microorganisms in recycled irrigation water and *Pythium* species indicate a deleterious effect on *Pythium* development with decreased numbers of zoospores released and suppressed formation of sporangia. These results suggest that microbial communities residing in recycled irrigation water may play a role in the suppression of *Pythium*.

Dispersal, infection and resistance factors affecting biological control of Canada thistle by *Puccinia punctiformis*

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The noxious weed Canada thistle, *Cirsium arvense*, causes extensive problems in pasture, landscapes and naturalized areas. Controlling Canada thistle with conventional management tactics is difficult due to the plant’s robust root system, aggressive growth and wind-dispersed seeds. The rust pathogen *Puccinia punctiformis* is a promising biological control agent that reduces Canada thistle infestations through fatal, systemic infections. Establishing the efficacies of *P. punctiformis* in Canada thistle requires a thorough understanding of the biology of both pathogen and host. To better understand the conditions under which epidemics can develop, we performed a series of experiments to evaluate dispersal characteristics of the various *P. punctiformis* spore types. Dispersal gradients were measured by releasing spores in windy field conditions and capturing spores at varying distances from the source. Terminal velocities of spores were also compared in a particle settling tower. By all measure, aerial movement of the two major types of *P. punctiformis* spores is significantly different. It is hypothesized that Canada thistle plants can have genetic resistance to some *P. punctiformis* lines. To investigated this possibility, we also compared resistance of thistle genotypes across Pennsylvania, which showed differential responses. Finally, to assess optimum timing and host tissue infection court, we evaluated the effects of season and placement of inoculum.

A disease of New Guinea impatiens (*Impatiens hawkeri* W. Bull) caused by *Pythium cryptoregulare*

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Root rot diseases caused by various species of *Pythium* are common in New Guinea flower crops produced in greenhouses in the northeastern United States. Diagnosis of the causal agent using morphological traits of the isolate is not precise in the case of *Pythium irregulare* because there are at least four cryptic species within the *P. irregulare* complex. One of those, *P. cryptoregulare*, is commonly found in greenhouses along with *P. irregulare sensu stricto*. A *Pythium* species has been consistently associated with New Guinea impatiens showing stunting, wilting and black vascular discoloration of stems and roots in NY since the 1980s. Isolates from discolored roots or stems of nine New Guinea impatiens clinic samples from 2003–2009 were identified as *P. irregulare* using morphological characteristics. Subsequent identification using ITS sequence data indicated that the DNA of all isolates matched that of *P. cryptoregulare*. Potted New Guinea impatiens plants that were lightly wounded and inoculated at the base with an 8 mm diameter plug from a corn meal agar culture of any of four *P. cryptoregulare* isolates showed stunted root systems and some root rot three weeks later. The oomycete was recovered on corn meal agar from 80–100 percent of root samples and 20–100 percent of stems of inoculated plants, but not from five control plants treated with plain agar plugs. The identity of the recovered pathogen was confirmed as *P. cryptoregulare* by ITS sequencing.

Use of Biochar to increase mycorrhizal colonization and suppress Fusarium crown rot of asparagus in replant soils

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Biochar (BC) is charcoal created by low temperature pyrolysis of biomass and offers new technology that sequesters and reduces atmospheric CO2. As a soil amendment, BC improves the soil structure and fertility, increases water-holding-capacity, and absorbs biological and synthetic toxins. Asparagus is highly susceptible to inhibitory allelochemicals (AC) that inhibit mycorrhizal associations (VAM) and increases susceptibility to crown rot caused by *Fusarium oysporum f. sp. asparagi* and *F. proliferatum*. *Fusarium*-infested soil that contained old asparagus roots was amended with BC at 0, 0.3, 1.5, or 3 biochar (w/w) and then planted with 2-mo. old susceptible asparagus plants. After 12 weeks, the percentage of diseased roots declined, and VAM colonization and plant weights increased with BC rate. In another study, three rates of AC (ferulic, coumaric, and caffeic acid) were drenched into soil, and suppression of root rot by BC improved the soil amended with a 0, 1.5, or 3% BC and planted. The addition of BC at both rates negated the damage caused by the BC and increased VAM. In a third study, dried, ground *Fusarium*-infested asparagus roots were added to soilless potting mix (12 g/liter) with and without biochar (3% w/w) and then planted with asparagus plants. The addition of the roots increased diseased roots from 60% in control to 45%, but the addition of 3% BC reduced this value to 25%. The data support the contention that biochar can be useful in restoring old asparagus fields back to productive use.
How the dynamics of plant disease epidemics depend on the timing of inoculum production

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The effect of latent period, infectious period and the temporal shape of the sporulation/reproduction curve on the course of the ensuing plant disease epidemic is examined using numerical simulation of disease development. The number, timing and size of lesion initiation and daughter lesion production (mean generation time) together with Vanderplank’s multiplication factor dominate the initial disease increase (exponential growth phase). Variation in the pathogen population about the mean generation time tends to shorten the effective generation time because lesions produced “early” tend to dominate the epidemic after a few generations. For most plant-pathogen systems, the peak of the sporulation curve is relatively unimportant and, after a few generations, the effective shape of the reproductive curve approaches a normal distribution. For some cases there is a long drawn out tail (positive skewness) of the reproductive curve due to “late” produced lesions which do not have time in a growing season to initiate multiple generations. The net effect of this is to reduce the effective inoculum multiplication factor with each successive generation.

Characterization of Pythium species causing Pythium blight of snap bean and other crops in the eastern U.S.

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New Jersey, Georgia, and the Eastern Shore of Virginia (ESV) are important snap bean growing regions. Profitable yields are threatened on an annual basis by Pythium blight, one of the most severe snap bean diseases in the U.S. Although the disease is well documented, the species of Pythium causing this disease on snap bean and other crops have not been well characterized. Knowing this information is important for determining proper management strategies. From 2008 to 2010, isolates were collected from the previously mentioned growing areas from different hosts, including snap bean, other legumes, cucurbits, and solanaceous crops to establish the causal agent(s) of Pythium blight. Isolates were collected from soil by baiting and from plant tissue showing water-soaking and/or white, cottony growth. For each isolate, pathogenicity on snaps beans was verified and the isolate was characterized by morphology and sequence analysis of the rDNA—internal transcribed spacer (ITS) regions. All ESV isolates were identified as Pythium aphanidermatum, with the exception of one P. myriotylum and four P. ultimum isolates. Both P. aphanidermatum and P. ultimum were recovered from New Jersey crops. P. aphanidermatum and P. ultimum were also isolated from symptomatic plants in Georgia, as well as multiple isolates of P. deliense. Due to the similarity of the ITS sequences of P. deliense and P. aphanidermatum, identification of P. deliense isolates was confirmed by PCR assays and North American Shrunken kernels and accumulation of mycotoxins (primarily deoxynivalenol or DON) are responsible for yield loss and economic loss. Barley spot blotch caused by Cochliobolus sativus is also responsible for yield loss and economic loss. This study examined the effects of Prosaro (Prothioconazole + Tebuconazole), Stratego (Propiconazole + Trioxystrobin), and Headline (Pyraclostrobin) on spring barley yields, DON control and barley spot blotch. Lygus bugs, and Fusarium—infected seed, seed germination, and bushel weight were not affected by fungicide treatment. All applied fungicides controlled barley spot blotch. Prosaro and Stratego applied at Feekes 8 or 10.5 did provide a statistically improved yield and an economic return compared to the untreated control. Headline did not statistically improve yield or provide an economic return compared to the untreated control.

New broad spectrum and safer fungicides for brown patch and dollar spot diseases of turf grass, powdery mildew of pumpkin and Septoria leaf spot of tomato

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Agion Technologies Inc. is developing highly effective fungicides/bacteria with levels of mixed metals up to 1,000 times lower than the earlier fungicides. Agion’s formulatons may function as a stand alone biocide or most valuable as a powerful synergist with conventional fungicide thereby reducing the use levels and their environmental impact, and has potential to show efficacy against the resistant strains. Agion formulations Agion-A, Agion-B, Agion-C and Agion-D significantly reduced disease severity over control in both Dollar Spot and Brown Patch field trials at Ohio State University (P = 0.05). There was no significant difference between these four
formulations and the standard fungicide, Daconil (P = 0.05). These formulations showed excellent efficacy in field trials conducted this year in Illinois against Septoria Leaf Spot of Tomato and Powdery Mildew of Pumpkins, where these formulations reduced significant disease severity over control (P = 0.05) and showed no significant difference in disease reduction over control compared to standard fungicides, Quadris and Bravo (P = 0.05). These results indicate that the Agion formulations have potential to be included in the IPM programs against several diseases in variety of different crops. Trials on other field crop, vegetables and fruit tree diseases are scheduled in upcoming seasons in 2011.

White pine needle diseases in eastern Canada

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In 2009, yellowing of white pine (Pinus strobus) needles was reported from several regions in three Canadian provinces: New Brunswick, Quebec and Ontario. A similar problem was seen also in eastern United States. Several causal agents were presented as hypotheses: drought, pollution as well as several needle diseases. In the spring and summer of 2010, samples of white pine needles were collected in areas where symptoms had been seen the previous year. Sampling was done by the three provincial agencies. In addition, samples were collected every month from September 2009 to August 2010 in Quebec City. At least six fungal species were observed or isolated from these needles. A few were parasites, some were endophytic fungi and were mostly obtained from diseased needles collected in June and some were secondary fungi like Henderosmia pinicola. The most common pathogen found was Canavirgella bandfieldii which is very similar to Lophophacidium dooksii. The yellowing of these needles was visible from late June, early July, 2010. The discoloration affects only the distal portion of the needles and not all of the needles in a fascicle are infected. Also, the lower section of trees seems to be more diseased than the top. Some white pines seem to be resistant to this disease. The teleomorph of C. bandfieldii appears on previous year needles in early summer. A second pathogen, Mycosphaerella dearnessii, has also been observed in June on previous year needles. The entire infec tion needle turns yellow and red bands are visible near the infection point. These needles drop a couple of weeks following their change of color. Both pathogens were often collected on the same tree. All these fungi are being sequenced and the results should clarify the synonymy of some fungal species and their classification at the family level.

Early season potyvirus epiphytotic affects cigar wrapper tobacco in Massachusetts and Connecticut

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In late June 2009, stunted shade and broadleaf cigar wrapper tobacco (Nicotiana tabacum L) plants in Massachusetts were observed with veinbanding, mosaic, and leaf mottling symptoms consistent with potyvirus infection. Plants tested positive for potyvirus, Potato virus Y (PVY), Tobacco etch virus and Tobacco vein mottling virus using independent ELISA tests (Agdia, Inc.). Two primers were designed to amplify a 400 nucleotide region between nucleotides 8200 to 8600 of PVY. RNA from symptomatic plants was used in a standard rtPCR reaction and amplified PCR product was sequenced to confirm PVY infection. Early symptom development and severity for tobacco near adjacent to test field crops. Approximately 1200 hectares of potatoes (Solanum tuberosum) were present in the affected area in Massachusetts and volunteer potato plants were found in high incidence in fields rotated from potato in 2008 to broadleaf tobacco in 2009. Growers indicated that volunteer tubers survived overwinter and emerged in large numbers in both 2008 and 2009. Minimum soil temperatures recorded 20 cm deep in Windsor CT were –4.7 C in 2007, and only –1.85 C in 2008. The minimum temperature required for potato tuber death are reported to be –2.8 C. Potato can serve as a virus reservoir, and volunteer tuber survival in two consecutive years likely increased early season virus incidence in 2010. Many species of aphids transmit these viruses in a nonpersistent manner. Affected tobacco was unmarketable as cigar wrapper and destroyed in the field. Crop insurance claims on losses were evaluated by adjusters and considered multiple causes, (e.g. 75% due to virus and 25% for excess precipitation). Over 255 hectares (nearly 20%) of shade and broadleaf tobacco production in Massachusetts and Connecticut were destroyed as a result of potyvirus infection at an estimated crop loss in excess of $10,000,000.

Efficacy of control methods on black rot caused by Xanthomonas campestris pv. campestris in greenhouse transplant production

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Black rot caused by the bacterium Xanthomonas campestris pv. campestris (Xcc) is often a serious disease in New York State crucifer fields. This pathogen is seed borne and even rigorous seed testing cannot guarantee every seedcarrying some xcc. A few of these transplants are ideal for the spread of Xcc with dense plant populations and overhead watering. Asymptomatic transplants can initiate field infections which are very difficult to control. We are investigating the efficacy of chemical and biological products that can be used to reduce the spread of Xcc in the greenhouse. Both a leaf wash (to determine CFU/g tissue) and real-time PCR were used to quantify pathogen numbers in asymptomatic seedlings. This comparison allowed us to identify treatments that were effective at suppressing Xcc in an environment favoring disease spread. Additionally, the role of plant age at time of control product application was studied to identify the most effective time to control black rot development in seedlings.

Usitago maydis as a model system for the study of a glycoprotein gene cluster in the biocontrol agent Pseudopezys flocculosa

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Pseudopezys flocculosa, an anamorph fungus member of the Ustilaginales, is an effective biocontrol agent (BCA) against several members of the Erysiphales. Its mode of action is still unclear but evidence suggests that the disease suppresses the fungus by the fungus promoting collapse of powdery mildew colonies. Interestingly, this molecule is quite similar to ustilagic acid, an antimicrobial metabolite produced by the model fungus Usitago maydis. Following the sequencing of P. flocculosa genome, we found that both flocculosin and ustilagic acid were under the control of a conserved gene cluster. Because P. flocculosa is not amenable to homologous recombination, we sought to use wild-type and mutant strains of U. maydis to carry out complementation studies in order to determine the role of flocculosin-specific genes. For each P. flocculosa gene complemented into the corresponding putative U. maydis mutant strain, overexpression of the gene restored the wild type phenotype as assessed through the structural analysis of ustilagic acid molecules produced by the U. maydis strains. Furthermore, fat3, a gene coding for an acetyltransferase specific to P. flocculosa, and assumed to account for an extra acetyl group in flocculosin was overexpressed in the U. maydis wild type strain FB1. We were thus able to make U. maydis produce a flocculosin-like molecule, i.e. with two acetyl groups instead of one, a structure never observed before in this fungus. Our results show that the U. maydis model system is well suited for the study of homologous proteins in P. flocculosa. With the imminent completion of the BCA genome assembly, one can thus expect that its annotation will be greatly facilitated by its close phylogenetic relationship with U. maydis and that these comparative analyses will shed new light on what triggers fungi to be pathogen or beneficial.

Anthracnose of Miscanthus sinensis caused by Colletotrichum graminicola

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Miscanthus sinensis Anders (Zebra grass, Maiden grass, Ulalia grass or Chinese silver grass) is an important ornamental grass species with yellow or gold strips on green leaves. Diseased leaves with anthracnose symptoms were collected from nurseries and landscapes in North Carolina and Tennessee in the fall of 2008. Typical leaf symptoms were fusiform, red-brown lesions with black acervuli formed in the light gray tissues. Fungal isolates from lesions were grown on half-strength potato dextrose agar at room temperature with a 12 h photoperiod. The fungal colonies were characterized by black conidiomata formed in ring on white vegetative mycelia. Setae were dark brown with three to five septa. Conidia were sickle-shaped with a range of 22.5 to 32.7 µm long and 3.1 to 5.6 µm wide. The average size of appressoria was 9.2 x 6.5 µm. These morphological characteristics are consistent with the description of Colletotrichum graminicola (Cesati) G.W. Wilson. Pathogenicity of the isolates was tested by spraying conidia suspension (10⁴ spores/ml) on adaxial surface of detached zebra grass leaf segments using a mini sprayer. Inoculated leaf segments were incubated on two layers of paper towels in a transparent plastic box at room temperature with 12 h photoperiod. Symptoms, observed 10 days after inoculation, were initially characterized by necrotic spots and then expanded as fusiform red-brown lesions with acervuli formed in the center of lesions. The fungus isolated from infected leaves and
showed the same morphological characteristics of the isolates previously inoculated. To our knowledge, this is the first report of C. graminicola infecting zebra grasses in North Carolina and Tennessee.

Can bacteriophage be used to control bacterial spot of peach?

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Bacterial spot of stone fruit, caused by the bacterium Xanthomonas arboricola pv. pruni (Xap), is an important disease of peach and nectarine in the northeastern U.S. Resistant cultivars have been marginally successfully. Control options are limited to copper compounds and antibiotics, which are problematic due to phytotoxicity and resistance. We are interested in determining if a naturally occurring bacterial virus (“phage”) can be used to protect plants from infection. Twenty-three Xap strains and 43 phage strains were isolated from 19 orchards in Connecticut, New York, and Massachusetts in 2009. An absence of genetic variability was demonstrated in both collections by infecting all bacterial strains with all phage strains; all bacterial strains were equally susceptible to all phage strains, and all phage strains were equally virulent to all bacterial strains. Three bacterial strains were then infected with ten viral strains. Surviving, putatively lysogenic, strains were isolated from resulting plaques. Each of the 30 lysogenic strains was then tested for susceptibility to all 43 phage strains, and the experiment repeated. Non-lysogenic (lytic) strains were used as positive controls. Because each lysogenic strain was resistant to all 43 phage strains, we concluded that all 43 phage strains were functionally, and therefore genetically, equivalent. This absence of phage diversity may pose problems for long-term sustainability as a bio-control, given that under laboratory conditions lysogeny occurs readily in this system.

Evaluation of combined effect of compost amendments and fumigation on strawberry verticillium wilt

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Phytopathology 101:S261

Strawberry (Fragaria × ananassa) is a valuable crop frequently affected by verticillium wilt caused by the fungus Verticillium dahliae Kleb. The disease causes wilting of mature leaves and can lead to plant death. Currently, pre-plant soil fumigation with costly chemicals is commonly used to control the disease. These chemicals, harmful for health and the environment, display variable efficiency and often lead to a negative shift in the biological equilibrium of the soil. Previous studies have shown that compost amendments may enhance plant growth and reduce symptoms of various diseases including verticillium wilt. In this context, the objective of this study was to assess the capacity of two composts and three fumigants to control strawberry verticillium wilt. To achieve this objective, V. dahliae naturally-infected field plots were fumigated with Vapam®, chloropicrin or Telone® C-17 or not (control) and subsequently planted with strawberry (cv. Seascape and Orléans). Then, the plots were amended with either bovine manure compost or marine residue compost at a rate of 40 t/ha or not (control). The results showed no significant difference in wilting incidence between fumigated and control plots and revealed a negative impact of fumigation on fruit yield in each individual plot. This result is in accordance with previous findings that compost amendments reduced the incidence of the disease and increased fruit yield in most of the treatments. The control of the disease apparently provided by the composts could in part be explained by the presence of beneficial microorganisms. This is supported by the isolation from the tested composts of several bacteria known for their antagonistic activity against plant pathogens.

Sensitivity of the cucurbit powdery mildew pathogen to fungicides prone to resistance development

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Podosphaera xanthii has a high potential to develop resistance to mobile fungicides with targeted activity, which are essential for effectively managing cucurbit powdery mildew. In 2009, 46 isolates were obtained from naturally infected leaves in the New York cucumber trial, predominantly near the end of the cropping season from fungicide-treated pumpkin in commercial and research fields on Long Island, NY. Their sensitivity to fungicides at risk for resistance was determined using a cotedyledon leaf disk assay. It was not feasible to test all isolates for all fungicides at all concentrations. Proportion of the isolates tested that were resistant (able to grow on at least half of the treated dishes) was 100% for 50 ppm thiophanate-methyl (FRAC Group 1 fungicide), 94% for 50 ppm trifloxystrobin (Group 11), 29% for 20 ppm myclobutanil (Group 3), 13% for 40 ppm myclobutanil, 44% for 50 ppm boscalid (Group 7), 10% for 500 ppm boscalid, and 23% for 10 ppm quinoxyfen (Group 13). Resistance to FRAC Group 1 and 11 fungicides is qualitative; therefore, isolates resistant to the dose tested would not be controlled by the fungicide applied to a crop (practical or field resistance). Resistance to Group 1 and 11 fungicides was extremely high. Resistance to the other FRAC groups is quantitative, thus a range of concentrations was tested and assay sensitivity needs to be compared to field application dose and efficacy to identify an assay dose corresponding to practical resistance. Isolates resistant to 500 ppm boscalid would exhibit field resistance because this dose is in the range of an application dose. Interestingly, 2 of 3 isolates resistant to 500 ppm boscalid that were tested at 40 ppm myclobutanil and 10 ppm quinoxyfen were found to also be resistant to these unrelated chemistries. Correlated resistance could challenge control.

Effectiveness for cucurbit powdery mildew of fungicides prone to resistance development

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Fungicides able to move from their deposition point are essential for managing powdery mildew effectively in cucurbit crops, especially large-leaved types like pumpkin due to the difficulty of delivering spray material directly to the lower surface where the pathogen (Podosphaera xanthii) develops best; however, mobile fungicides are at risk for resistance development due to their single-site mode of action. A replicated field experiment was conducted with pumpkin in 2010 to examine efficacy of the fungicides and determine if fungicides are at risk for resistance when this disease. Efficacy of each fungicide applied at the highest label rate was compared to efficacy at lower rates and/or efficacy in previous years. Lack of consistent control could be an indication of resistance. Degree of control achieved on upper and lower leaf surfaces based on AUDPC values was 94% and 95%, respectively, for Quintec (active ingredient quinoxyfen; FRAC Group 13) at high label rate, 84% and 50% for Procure (triflumizole; Group 3) at middle label rate, 94% and 69% for Procure at high label rate, 75%* and 31%* for Pristine (boscalid and pyraclostrobin; Groups 7 and 11) at low label rate, and 68% and 50%* for Pristine at high label rate (*AUDPC value not significantly different from non-treated control). Efficacy of Quintec and Procure was similar in 2009 and 2010. Pristine at high rate was more effective than Quintec in 2009 and 2010: 98% and 80% control on upper and lower leaf surfaces, respectively, in 2009. Resistance could account for detected deviations in efficacy for Procure and especially for Pristine. Strains of the pathogen resistant to Group 11 fungicides have been common in recent years while resistance to Group 7 chemistry is a new development.

The sensitivity of Colletotrichum cereale to in vitro exposure with Velis™ (penthiopryad)

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The fungicide Velis™ (penthiopryad) is a new active ingredient in the carboximide/SDHI class of fungicides being registered by DuPont Professional Products for use on golf courses and intensively managed turf. This fungicide has previously demonstrated activity against anthracnose (Colletotrichum cereale) in experimental field trials. In order to determine how well it controlled the pathogen in a controlled environment, in vitro petri dish assays were undertaken to examine its efficacy compared to 8 other commercially available turfgrass fungicides. Penthiopryad was tested on 27 C. cereale isolates collected from the Northeast United States between 1993 and 1995 and 23 isolates collected from the same region in 2007. When compared to azoxystrobin, chlorothalonil, fluazinam, iprodione, polyoxin-D, thiophanate-methyl and triadimenol, penthiopryad produced the lowest mean log transformed EC50 values of any chemical examined; 0.31 µL/ml and 0.19 µL/ml, from the two respective isolate collections. Of the other fungicides tested, only polyoxin-D even produced an EC50 below 4.0 µL/ml and only for the 1993–1995 isolate collection. In addition to its activity against C. cereale, penthiopryad has performed extremely well in field trials against dollar spot (Sclerotinia homoeocarpa) and brown patch (Rhizoctonia solani) and may provide an effective, broad spectrum fungicide for simultaneous use on a wide array of turfgrass pathogens.

Characterization of Phytophthora infestans isolates from potato/tomato in 2010

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Late blight in New York and apparently also in the Northeast was much less a problem in 2010 than in 2009. Nonetheless, our lab received more than 70 samples originating from: Connecticut, Kentucky, Louisiana, Maine, Maryland, Massachusetts, New Hampshire, New York, Pennsylvania, and Wisconsin. In New York, we received samples from (Broome Chenango, Erie, Genesee, Livingston, Madison, Niagara, Suffolk, Tioga, Tompkins, Washington, Yates Counties). Samples were also received from Ontario, Canada. Characterization of confirmed *Phytophthora infestans* isolates was based upon, the protein profile, mating type, DNA genotype, resistance to metalaxyl/mefenoxam and pathogenicity of the isolate. The majority of samples were obtained from tomatoes and our preliminary results suggest that most of these belonged to the US22 clonal lineage – the new tomato strain that was epidemic in the Northeast in 2009. US22 has been largely sensitive to metalaxyl/mefenoxam and appears to be somewhat more aggressive on tomato compared to potato. From our samples, the only occurrence this year of US22 on potatoes was from a site in which the potatoes were adjacent to tomatoes that were infected with US22. US8 was also detected, but only on potatoes – in Yates and Wayne Counties in New York, and in Ontario, Canada. US8 is very aggressive on potatoes, does very little to most tomato cultivars and is resistant to mefenoxam. We also found a few (at least three) “rare” and as yet uncharacterized genotypes of *Phytophthora infestans*. Two of these were from tomato, and one from potato.

**Post-harvest foliar urea sprays as an effective sanitation practice for reducing ascosporic production by *Venturia inaequalis***

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Apples are an important crop in the Northeast and apple scab, caused by *Venturia inaequalis*, is the most important disease of apples. Apple scab is typically managed by applying frequent applications of chemical fungicides in the spring to prevent infection by ascospores, the only significant source of primary inoculum. We examined the use of post-harvest foliar urea sprays to reduce the production of ascospores by *V. inaequalis*. Single and split applications of 5% urea were sprayed by hand onto naturally-infected ‘Cortland’ leaves in the autumn of 2007, 2008 and 2009. Urea applications were made immediately after harvest, at the start of leaf fall and at 95% leaf fall to determine the most effective time of application to reduce ascospore production. Single 5% urea applications were compared to two 2.5% and three 1.6% split applications to determine if multiple applications were more effective than a single 5% application. In 2008 and 2009, an additional treatment of two 5% applications was added. The same urea treatments were applied with an air blast sprayer to ‘Marshal Mac’ trees in 2007, 2008 and 2009 to determine the effects of the above treatments on winter injury, fruit set and foliar nitrogen content. Our results indicate that all our urea treatments significantly reduced ascospore production while having no adverse effects on winter injury, fruit set and foliar nitrogen content.

**Detection of propiconazole field resistant *Sclerotinia homoeocarpa* isolates**

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Dollar spot (*Sclerotinia homoeocarpa*) is a major turfgrass disease requiring multiple fungicide applications to maintain acceptable turf quality each year. The demethylation inhibitor fungicide class (DMI) is frequently used and resistance to *S. homoeocarpa* has been confirmed. The objective of this study was to determine the in vitro propiconazole (DMI) sensitivity of field resistant isolates of *S. homoeocarpa*. Isolates were sampled from five locations (HGC, HRC, TFC, SMCC, and WBGC) before propiconazole treatment (0.44 kg a.i. ha⁻¹) and seven days after treatment (DAT). Propiconazole sensitivity was determined by calculating the relative mycelium growth (RMG) percentage of all isolates on potato dextrose agar amended with 0.1, 0.3, 0.5, and 1.0 ug a.i. ml⁻¹ of propiconazole. Isolates sampled from active or newly infected *S. homoeocarpa* infection centers seven days after propiconazole treatment were considered field resistant isolates since propiconazole is labeled for a minimum of 14 days control. Field resistant isolates were sampled from the WBGC, SMCC, HGC and HRCC sites, but not from JTRF (lack of infection 7-DAT). Ninety five percent of field resistant isolates ranged from 50–100% RMG on 0.1 ug a.i. ml⁻¹ and 99% of field resistant isolates were capable of growth on 1.0 ug a.i. ml⁻¹. Relative mycelium growth ranged from 0–40% on 0.1 ug a.i. ml⁻¹ for all isolates sampled from JTRF and no growth was observed on 1.0 ug a.i. ml⁻¹. Results indicated that *S. homoeocarpa* isolates with RMG values above 50% on 0.1 ug a.i. ml⁻¹ of propiconazole or growth on 1.0 ug a.i. ml⁻¹ of propiconazole are capable of causing infection seven days after application in the field. This qualitative sensitivity assay may be useful for DMI resistance monitoring of *S. homoeocarpa* populations.

**Evaluation of Juglans cinerea trees putatively resistant to butternut canker (Sirococcus clavigerrnti-juglandacearum): A new project supported by the Canadian Interdepartmental Recovery Fund**

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*Sirococcus clavigerrnti-juglandacearum* is considered the major pest of butternut survival. It is threatening its native range, which extends from New Brunswick to Georgia and west to Minnesota and Arkansas. In Canada, butternut was listed as endangered under the *Species at Risk Act* in 2005. A project aimed at identifying, propagating and testing trees putatively resistant to butternut canker is being funded by the Interdepartmental Recovery Fund (Environment Canada). As butternut is shade intolerant, another goal is to release some trees in order to improve their vigour and, hopefully, their resistance to butternut canker. To date, 146 putatively resistant trees have been located on seven sites in the province of Quebec. These trees had to have at least 50% of live crown and canker-free stems or more than 70% of live crown with less than 25% of the combined circumference of the bole and root flares affected by cankers. Compared with a survey conducted from 2006 to 2008, current damage appears more severe on these sites. As butternut seeds are able to disperse the pathogen, efforts will be made to vegetatively propagate the selected trees. Twigs from these butternuts will be collected and assays will be carried out to obtain plantlets from axillary bud cultures. In case of failure, the literature indicates that propagation is also possible using cutting, grafting or somatic embryogenesis techniques. Once the vegetative material is obtained, resistance tests will be conducted in a greenhouse because the pathogen will be added on susceptible trees will serve as control. Should resistant trees be found, they will be propagated and, thereafter, some material will be confronted against the pathogen to improve its resistance while other material will be used to restore some sites, thus giving back to this valued species the status it entirely deserves.

**Non-target effects of glyphosate on apples**

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Phytopathology 101:S262

Since 2004, apple trees in scattered orchards in southeastern New York have developed trunk cankers extending upward from the soil line. Cankers on *Macon*’ trees, the most severely affected cultivar, caused tree decline and death. Orientation of cankers suggested herbicide residues were a contributing factor. Glyphosate had been used in all affected orchards. However, Macoun trunks sprayed with glyphosate in 2007 as part of a replicated trial did not develop cankers, so etiology of basal trunk cankers remains uncertain. In another trial, ‘Empire’ fruit from trees exposed to simulated glyphosate spray drift during summer of 2009 developed more internal browning, a physiological disorder, after eight months of controlled atmospheric exposure than fruit from control trees. Four mature trees on each of three farms were exposed to glyphosate by applying Roundup PowerMax® at a concentration of 0.26 mL/l to one or several lower limbs on each tree. The number of limbs or shoots treated was determined by the need to collect 25 fruit from the sprayed limbs at harvest, but the canopy area exposed to glyphosate never exceeded 30% of leaf area on lower scaffold limbs. Fruit samples were harvested in early October from both treated limbs and from untreated upper limbs of both sprayed trees and unsprayed control trees. Samples were stored for eight months at 2.2°C with carbon dioxide and oxygen held at 2%. They were then held at 20°C for 7 days before they were evaluated for internal browning. Incidence of internal browning for fruit from glyphosate-exposed trees from the three farms was 22, 61, and 133% greater than for fruit from control trees. Browning was more severe in fruit from the tops of treated trees than in fruit directly sprayed with glyphosate. Thus, glyphosate exposure may affect the incidence and severity of internal browning in stored apples.
Aerial dispersal of Phytophthora infestans as a component of a Late Blight Decision Support System
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Phytopathology 101:S263

A late-blight dispersal-risk algorithm capable of determining favorability of weather conditions for sporulation, dispersal and survival of spores, and subsequent infection of host tissue has been developed. The influence of weather conditions on these processes was obtained from published and unpublished data. The algorithm uses temperature, relative humidity, wind speed and direction, as well as solar radiation. Historic (observed) data as well as forecast data are used. For each potential risk period independent indices are calculated for sporulation, dispersal and survival of sporangia, as well as for subsequent infection of target host tissue. Proximity of the target to an inoculum source (if present) may be utilized in the risk index at the discretion of the user. These indices are then integrated to provide an overall risk index. Because the algorithm uses future weather as well as historical weather, it enables users to take precautionary measures. Preliminary experiments suggest that forecast “high risk” periods could be used to enhance the efficiency of disease management practices. The Decision Support System is structured to enable the communication of “inoculation alerts” to users.

Colonization of Peronospora belbahrii by the basidiomycetous yeast, Pseudozyma
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Phytopathology 101:S263

Downy mildew of basil, caused by Peronospora belbahrii was first reported in the United States in 2007. During 2008 and 2009 downy mildew of basil was in epidemic proportions from Florida to Massachusetts resulting in losses up to 100 percent in both greenhouses and in the field. A specimen of downy mildew received from Georgia had conspicuous mycelial growth covering the sporangiophores and sporangia. Isolations from the growth yielded a yeast. When the yeast was inoculated to downy mildew on basil leaves, a mycelial colony developed over the downy mildew. Sequence analyses were conducted on three isolates of the unknown yeast. PCR amplification of the ITS-1, 5.8S, and ITS-2 region was performed using universal primers ITS6 and ITS4. In addition, a roughly 0.9 kb amplicon was produced from the 5′ end of the nuclear large subunit (nLSU) using universal primers LROR and LR5. The sequences of the three isolates were identical from both regions. BLAST analyses of the sequences revealed a 99% similarity to Pseudozyma aphidis. Nutritional utilization and morphology of the isolates were also consistent with the genus Pseudozyma aphidis. Of 13 morphologically similar yeasts inoculated to basil downy mildew, some did not colonize and several showed vigorous growth over the sporangia. Observations of basil downy mildew from CT and MA in 2010 indicated that filamentous yeast commonly occurs on Peronospora belbahrii. An investigation of the yeast/downy mildew relationship, and a survey for more vigorous yeast isolates are underway.
Development of a rapid laboratory screening method for stem rot resistance in peanut
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Phytopathology 101:S264

Stem rot (caused by Sclerotium rolfsii) is the most important disease of peanut (Arachis hypogaea) in Georgia. Infection involves the release of oxalic acid (OA) exudates to break down cell walls preceding mycelial colonization. Thirteen peanut cultivars grown in the greenhouse and field were screened for stem rot resistance in the laboratory by either placing 200 mM OA droplet solution onto detached leaves, dipping the basal tip of excised stems into 100 mM OA solution, or inoculating the main stems, pods and pegs with mycelial plugs of S. rolfsii. Pearson correlation coefficients were used to compare the disease response with laboratory screening methods to three years of field evaluation data. Differences in reaction to OA, measured as lesion length on stems for four consecutive days after treatment, were correlated (r = 0.9448, P = 0.0051; r = 0.6803, P = 0.0303; r = 0.5918, P = 0.0527; r = 0.4236, P = 0.0648, respectively) with the field data, but the correlation of leaf response to OA and field data was not significant. Cultivar responses to inoculation of the main stem with S. rolfsii were correlated (r = 0.6823, P = 0.0339) with the field screenings, but correlations between pod and peg inoculation methods and the field data were not significant. The results suggest that both OA dipping and S. rolfsii inoculation of main stems could rapidly screen peanut germplasm for stem rot resistance.

The roles of light induced proteins in the biosynthesis of cercosporin by Cercospora kikuchii
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Phytopathology 101:S264

Cercospora leaf blight (CLB), caused by Cercospora kikuchii, has recently emerged as a serious threat to soybean production in the Unites States. It is the causal agent of soybean purple seed stain. Most soybean cultivars are susceptible to C. kikuchii, and the fungicides have not been efficacious in controlling CLB disease. C. kikuchii produces a perylenequinone photo-sensitizer known as cercosporin, which is known to play a critical role in pathogenicity and virulence of C. kikuchii in soybeans. With the use of two-dimensional gel electrophoresis, several C. kikuchii proteins were specifically up-regulated in cultures grown under light. Included among these were hydroxynaphthalene reductase and adenosylhomocysteinase. Their corresponding genes were cloned from C. kikuchii, and disruption mutants are being produced to characterize the involvement of these proteins in cercosporin biosynthesis. The resulting C. kikuchii mutants will be tested for cercosporin production and for changes in pathogenicity or virulence on soybean.

A genomics study of Burkholderia glumae genes regulated by quorum sensing
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Phytopathology 101:S264

Burkholderia glumae is the major causal agent of an economically important rice disease. Bacterial panicle blight (BPB). The known virulent factors of B. glumae, toxoflavin, fagella, lipase and catalase share a LuxI/LuxR-type quorum sensing system as their regulator. tofI and tofR genes encode the N-acetyl homoserine lactone (AHL) synthase for the B. glumae quorum sensing signals, N-octanoyl homoserine lactone (C8-HSL) and N-hexanoyl homoserine lactone (C6-HSL), and the receptor for C8-HSL, respectively. Even though B. glumae produces both C6-HSL and C8-HSL, the function of C6-HSL is still unknown. According to sequence information from the National Center of Biotechnology Information (NCBI), there are at least six putative LuxR homologs in the entire genome of the B. glumae strain BGR1, but they are not coupled with LuxI homologs. Therefore, C6-HSL may involve in the regulatory network through other LuxR-family proteins other than TofR. In this study, B. glumae genes dependent on different autoinducers and regulators of the B. glumae quorum sensing system were sought using various techniques of molecular genetics and genomics. Though this study, we expect that the quorum-sensing network for regulating the virulence of B. glumae would be better understood.

Seed treatment using plasma technology in control of seedborne diseases
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Phytopathology 101:S264

Novel seed treatment was evaluated using plasma that is a state of matter similar to gas with ionized particles. Cold plasma generated by dielectric barrier discharge (DBD) in atmospheric pressure was applied on agar plates and rice seeds inoculated with a seedborne pathogen: Fusarium moniliforme (causal agent of bakanae disease) or Burkholderia glumae (causal agent of bacterial panicle blight). The cold plasma treatment inhibited growth of the pathogens on agar plates, and significantly reduced the number of colony forming units (CFU) of both pathogens on seeds. Significant CFU reduction of F. moniliforme occurred after 30 sec or longer exposure by the cold plasma. However, seeds treated with plasma did not show any abnormal conditions on viability, germination and seedling development. This study indicates that application of cold plasma can be a valuable disinfection technique for the removal of seedborne fungal and bacterial pathogens on the surface of the seed.

Histochemical analysis of wheat resistance to leaf blast mediated by silicon
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Phytopathology 101:S264
Blast, caused by *Pyricularia grisea*, is the most important disease of wheat in Brazil and is difficult to control. Since previous research has shown that silicon (Si) suppresses a number of plant diseases, especially in monocots, the purpose of this study was to determine if Si could enhance wheat resistance to blast and obtain some insights as to why. Disease severity was 49% greater on non-amended plants (Si-) in comparison to Si amended (Si+) plants. Tissue at the infection sites was bright yellow under UV light for both Si+ and Si- plants, however, the fluorescence was more intense in tissues of Si+ plants. Necrotic tissue also showed the same hue and intensity fluorescence in both treatments (Si+ and Si-), suggesting that the fluorescence is due to the same compound or group of compounds. Hyphal colonization was found in leaf tissue of Si- plants, but in Si+ plants, fungal hyphae were found in no more than two epidermal cells. Furthermore, signals of cytoplasm granulation were only observed in the epidermal cells of Si+ plants. Results from this study demonstrated that Si enhanced wheat resistance to blast and was probably potentiates by the metabolic activation of phenyl-propanoid.

**Management strategies for Pythium pod rot of peanut in Oklahoma**

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Phytopathology 101:S265

Pythium pod rot of peanuts was widespread in Oklahoma during 2010 and most severe on Virginia market-type cultivars. Fungicide programs, calcium sulfate application, and cultivar resistance were evaluated as disease management strategies. Fungicide programs, consisting of applications at pegging and pod set (60 and 90 days after planting) were compared on the \textit{Virginia-type} cultivar “Jupiter”. Disease incidence (DI) was severe, exceeding 60% in untreated plots. Plots treated with a phosphorous acid (DI = 39%) and phosphorous acid + azoxystrobin (DI = 29%), but not mefenoxam, azoxystrobin + mefenoxam significantly (\(P = 0.05\)) reduced disease incidence compared to the untreated control. Over all plots, disease incidence was negatively correlated with yield (\(r = -0.47,\ P = 0.02\)). All treatments except mefenoxam increased yield compared to the untreated control. Yield responses ranged from 691 kg/ha for phosphorous acid to 1038 kg/ha for azoxystrobin + mefenoxam. Calcium sulfate was applied at pegging at rates of 0, 560, 1120, and 1680 kg/ha in an adjacent trial on the same cultivar. Treatment effects on pod rot were not significant (\(P = 0.3\)) and disease incidence ranged from 47% for the 0 kg/ha treatment to 57% for 560 kg/ha treatment. In evaluations of cultivars and breeding lines where pod rot was severe, Virginia types (DI = 38%) were more susceptible (\(P = 0.05\)) than Spanish (DI = 17%) and runner (DI = 21%) types. One or more entries within each market type had <10% pod rot. Planting resistant peanut cultivars was more effective than fungicide programs for control of Pythium pod rot, while application of calcium sulfate was not effective.

**Utilizing nematode resistance in cotton production**

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Phytopathology 101:S265

In the US, losses to plant-parasitic nematodes are especially severe in cotton where root-knot (\textit{Meloidogyne incognita}) and reniform (\textit{Rotylenchulus reniformis}) nematodes each typically cause greater losses nationwide than any other single pathogen. Damage from nematodes is likely to become even more significant in cotton because the predominant nematicide, aldicarb, is being phased out in the US. Cotton gernplasm that is highly resistant to \textit{M. incognita} was first created in the 1960s, but highly resistant cultivars have not yet been developed. A high level of resistance to \textit{M. incognita} in cotton is a multiphenic trait and has proven difficult to maintain in breeding programs. Resistance was recently introgressed from another \textit{Gossypium} species. Sources of resistance to \textit{M. incognita} and \textit{R. reniformis} in available germplasm are very limited. Ongoing research has identified DNA markers for two chromosomal regions imparting resistance to \textit{M. incognita} and one region imparting resistance to \textit{R. reniformis}, thereby advancing the possibility of marker assisted selection, which is widely believed to be a prerequisite for commercialization of resistant cotton cultivars. In the absence of resistance, both the absolute and percentage yield suppression in cotton caused by \textit{M. incognita} increase as yield potential increases, which indicates that resistance will have proportionally greater benefit in high-yielding cultivars. Although a high level of resistance is preferable, moderate levels of resistance have been shown to contribute significantly to nematode suppression in the field.

**New chemistry and MI gene for root-knot nematode management in vegetables**

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Phytopathology 101:S265

There are several new candidate nematicides under development for managing pathogenic nematodes in vegetables. Unfortunately none have given efficacy performances that would suggest they would be attractive candidates for use in high value crop production. The root-knot nematode tomato cultivar, Cistuba was tested for marketable yield and galling on roots in two spring and two autumn trials. Evaluations included fumigated and nonfumigated treatments and all trials included root-knot nemadite susceptible cultivar. In three of these trials fruit yield with nonfumigated Cistuba ranged from 33% to 113% greater than that of the nonfumigated susceptible cultivars, whereas in one trial fruit yield of Cistuba was 17% less than that of the susceptible cultivar. In three of the four trials when plots of Cistuba were treated with various fumigants (chlororopicrin, potassium metam, 1,3-D, 1,3-D-chlororopicrin) yield was increased from 7 to 20% over the nonfumigated control; however, yield of Cistuba was decreased 21% when fumigated compared with nonfumigated Cistuba. Gailing indices on nonfumigated Cistuba ranged from 0 to 2%, whereas galling on nonfumigated susceptible cultivars ranged from 29 to 53%. The low percentage galling of Cistuba vs. susceptible cultivars indicate that the root-knot nematode resistant \textit{Mi-I} gene in Cistuba was maintained and not broken by soil temperature.

**Infestation of ovules by \textit{Acidovorax citrulli} during pistil and pericarp invasion in developing watermelon fruit**

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Phytopathology 101:S265

Infestation of watermelon ovules by \textit{Acidovorax citrulli} during pistil and pericarp invasion was compared in the early phases of seed development. Under greenhouse conditions, the stigmas of root-knot nematode-infested female watermelon blossoms were hand pollinated followed by inoculation by depositing 10 µL of \textit{A. citrulli} suspension (10^6 CFU mL^{-1}) onto stigmas or swabbing the pericarp of the ovary with the same level of inoculum. Blisters inoculated with 0.1M phosphate buffer saline were used as negative controls. Immature ovules were taken from each ovary at 24 h, 48 h, 72 h, 96 h, and 168 h after inoculation. Samples, comprised of 15–20 ovules from three watermelon ovaries inoculated either through the pistil or the pericarp, were tested for \textit{A. citrulli} by dilution plating on semi-selective medium. After incubation for 3 days at 28°C the mean proportion of \textit{A. citrulli}-infested ovules was determined and analyzed. Concurrently, at each time point, bacterial ingress was observed microscopically for each blossom treatment. In the independent experiments, significantly higher proportions (\(P < 0.05\)) of \textit{A. citrulli}-infested ovules were observed with pistil (23.13%) than pericarp (4.17%) infection. Microscopy showed that during pistil-invasion, \textit{A. citrulli} colonized the stigma, style, and ovary by 24 h post-inoculation. In contrast, with pericarp-invasion, \textit{A. citrulli} was observed in epidermal and sub-epidermal tissues of pericarp and failed to colonize pulp/flesh of the ovary during the study period. These results suggest that watermelon ovules become infested with \textit{A. citrulli} earlier in seed development by pistil as compared to pericarp invasion.

**The use of foliar fungicides did not improve yields in wheat in the absence of wheat leaf rust or other foliar diseases in Texas: 2008–2010**

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Phytopathology 101:S265

Wheat leaf rust, caused by \textit{Puccinia triticina}, is the most important disease of wheat in Texas. Foliar applications of fungicides have been a major component in disease management in Texas. Wheat varieties TAM 111, TAM 112, TAM 304, and Fannin, received an application of a fungicide (azoxystrobin + propiconazole) at full head emergence (Feekes 10.5) in two field plots in the Texas panhandle for the 2008, 2009, and 2010 growing seasons. One location was under irrigation while the other was non-irrigated (dryland). Both locations were free of leaf rust or any other disease for at least three years at the time of spraying and only trace levels of rust were observed by the end of all growing seasons. No significant differences in yield were observed between plots that received or did not receive a fungicide application, regardless of variety and for all three years. In the 2008, a similar trial conducted in the Texas Rolling Plains with the same varieties and fungicide treatments also had no differences in yield for any variety. However, an additional treatment with a split application of the fungicide a week later had a significant difference in yield for TAM 111 only. Although no foliar diseases were observed, this variety did have an incidence of around 5% of Fusarium root rot (dryland foot rot). In plots where only azoxystrobin was sprayed, no differences in yield were observed in the absence of foliar diseases. In other locations in Texas where wheat leaf rust was present in significant amounts, fungicide applications were effective in managing this disease. In the absence of leaf rust or any other fungal disease in wheat, a fungicide application was not warranted for these locations tested.
Molecular genetic and genomic studies on bacterial panicle blight of rice and its causative agent Burkholderia glumae

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Phytopathology 101:S266

Burkholderia glumae is the chief causative agent causing bacterial panicle blight (BPB) in rice. Outbreaks of BPB have resulted in severe yield losses in the southern United States including Texas, Arkansas, and Louisiana. Favorable high temperatures, rice crop problem in East and Southeast Asia and Central America probably due to the current global warming. Despite its economic importance, virulence mechanisms of B. glumae are poorly understood compared with that of other important plant pathogenic bacteria because the BPB research is currently in its early stages worldwide. Our research group has been performing molecular genetic and genomic studies on the pathogen B. glumae and the rice-B. glumae interactions. In particular, several novel regulators including the TepS/TepR two-component regulatory system that control the expression of B. glumae virulence genes were identified and a new NAC4-like transcription factor of rice associated with the partial resistance to BPB was discovered from our study. Currently, comparative genomic studies and transcriptome analyses of B. glumae using a high-throughput sequencing technology are also being undertaken for genome-wide overview of genomic diversity based on geographic origin and transcriptional dynamics depending on bacterial quorum-sensing. New findings obtained from these research activities on this emerging rice disease will be presented. In addition, our current efforts on genetic mapping of the partial resistance to BPB and breeding new disease resistant rice lines will be introduced.

Site specific management of nematodes in multiple soil types with Telone® H in Louisiana

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Phytopathology 101:S266

Root-knot nematodes (Meloidogyne incognita) and reniform nematodes (Rotylenchulus reniformis) are serious pest species of cotton which often occur in the same field. Choosing the most appropriate nematicide program is complex and requires the understanding of crop rotation, nematode species present, soil types, nematicide application and efficacy, economics, etc. Research projects in LA, AR, GA and SC have addressed development and implementation of risk management zones based on soil type and/or apparent soil electrical conductivity (ECa). Root-knot nematodes cause more damage in low-density sand soils with higher ECa than in heavier clay soils with higher ECa values. Thus, root knot nematode infested fields lend themselves well for site-specific applications of nematicides. Research results show that the use of soil maps and ECa data can be used effectively to identify low and high risk or “responsive” zones and that input costs can be reduced by 30–40%. However, since reniform nematodes can thrive in soils with higher clay content, the application of the nematicide becomes more challenging in fields with variable soil types infested with more than one species of nematode. This challenge was the basis for this research project conducted in a field with 3 distinct soil types that were infested with both root knot and reniform nematodes. Strips of Telone® Hl were applied across the different soil zones in 2009 to better define the responsive areas of the field. Yield monitor data from this trial was then used in conjunction with ECa data and soil maps to build a prescription for the 2010 season. Verification strips were also embedded in the 2010 study and clearly demonstrated the effectiveness and value of the nematicide in the three soil types with varying ECa ranges. *Trademark of Dow AgroSciences LLC. Telone is a Restricted Use Pesticide.

Yield losses due to southern corn rust in Louisiana: A preliminary view

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Phytopathology 101:S266

Southern corn rust (SCR) is a disease that historically occurs late in the growing season in Louisiana. Estimates of yield losses are low due to its late appearance. When SCR does appear earlier in the season, yield losses are estimated at <5%. Even though losses estimates are made annually, accurate measurements have not been taken. In this study, fungicides were used as a tool to influence SCR development in small plots. Applications were made with the aid of CO2-pressurized sprayers delivering 150L/ha of Headline AMP (1.05 L/ha) solution at VT (tasseling) +14d +28d to stop or delay disease development to determine the effect of disease development on yield. At the Ben Hur Research Farm three Pioneer Brand hybrids were used with differing levels of SCR susceptibility. Yield losses were measured at 11.4% on Pioneer 31G71 [0 to 20.75% leaf area coverage (LAC) with SCR], 13.8% on Pioneer 31D59 (1.25 to 23.50% LAC with SCR) and 20.3% on Pioneer 33F87 (0 to 18.75% LAC with SCR). On a nearby commercial farm using the same hybrids, losses were 19.2%, 21.1% and 23.3%, respectively. At another commercial farm, a single fungicide application at R5 (early dent) was done on Pioneer 31D59 resulting in a 6.1% loss (6.75 to 7.50% LAC with SCR) compared to the untreated.

Variation in virulence among isolates of Phytophthora nicotianae recovered from Catharanthus roseus

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Phytopathology 101:S266

Phytophthora nicotianae is one of the most economically important pathogens of the bedding plant Catharanthus roseus (Madagascar periwinkle or annual vinca). It routinely causes stem and foliage blight and occasionally may cause root rot; infection usually results in mortality. Several years ago, the Coral series of C. roseus was introduced as resistant to P. nicotianae, but several instances of susceptibility to this pathogen were noted. Consequently, three cultivars of C. roseus, two resistant (Cora Lavender, Cora Burgundy) and one susceptible (Titan Blush), were used to evaluate the virulence of 40 isolates of P. nicotianae that had been recovered from C. roseus over a 13-year period—including four isolates from diseased Cora plants. All isolates also were tested for mating type and sensitivity to mefenoxam. Isolates varied significantly in virulence, cultivars varied significantly in susceptibility, and there was a significant isolate-by-cultivar interaction—indicating that isolates affected cultivars differentially. Isolates were separated into three virulence groups: weakly, moderately, and highly virulent. Nine weakly virulent isolates caused little disease on any of the plants, and 21 moderately virulent isolates caused disease primarily on Titan Blush plants. The 10 highly virulent isolates caused disease on plants of all three cultivars—often killing both Titan and Cora plants. Highly virulent isolates were mefenoxam sensitive and usually mating type A2.

Role of melanin-like brown pigments in the virulence of Burkholderia glumae

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Phytopathology 101:S266

Burkholderia glumae is the major causative agent of bacterial panicle blight (BPB) of rice whose growth and pathogenicity is favored by high temperatures. BPB is becoming a serious threat in rice-producing areas around the world; however, not much has been studied about this disease and the role of melanin-like pigments of B. glumae. So far phytotoxin (toxoafavin), cell wall degrading enzymes (lipase and polygalacturonase), motility driven by flagella, and catalase for protecting bacterial cells from visible light have been known to be involved in virulence and an LuxI/LuxR-type quorum-sensing system is known to be a global regulatory system that controls these virulence factors. Recently, we found that some of the B. glumae strains produce melanin-like pigments. The melanin produced by microorganism is related to virulence by reducing the host’s antimicrobial resistance mechanism, scavenging superoxide radicals, and providing resistance to UV light. In an attempt to identify the role of the melanin-like brown pigments in virulence, the genome of B. glumae strain 411gr-6 was randomly mutagenized with a mini-Tns5 derivative, mini-Tns5gs. Mutants showing altered phenotypes in the production of the melanin-like pigments were screened and mutated genes were subsequently identified. With these mutants, the role of melanin-like brown pigments in virulence and its regulatory system for the pigment production in B. glumae were identified.

Anticipated management tools for nematodes affecting agronomic crops through 2015

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Phytopathology 101:S266

Nematodes cause significant losses to agronomic crops in the southern U.S. and include Meloidogyne incognita, M. arenaria, Rotylenchus reniformis, Holopeltis Columbus, Heterodera glycines, Paratrichodorus minor and other plant-parasitic nematodes. In addition to rotation with non-host crops, management of nematodes affecting peanuts and cotton has included aldicarb and 1,3-dichloropropene. Management of cyst and root-knot nematodes on...
soybean centers on host resistance and use of a nematicides. Management on corn has been less important than on other crops; growers in the southeastern U.S. now have greater interest in nematode management on corn. Management of nematodes affecting these crops will undergo significant changes in the next 5 years and beyond. Seed-treatment nematicides continue to expand beyond cotton, corn and soybeans to new crops and the introduction of new crops. Loss of aldicarb will result in evolving use of nematicides like 1,3-dichloropropene and oxamyl and availability of new products necessitating careful assessment and evaluation. Release of cultivars with resistance to nematodes, e.g. 'Tifguard' peanut, Phytoxy 367WRF cotton and Stoneville 54SBBRF cotton will provide new tools. Management of nematodes through 2015 will integrate variable rates of fumigants like Telone II with use of risk management zones, further adoption of seed-treatment nematicides, and integration of varieties with increased resistance.

The duration of DMI exposure affects the expression of ShCYP51 differently in sensitive and insensitive isolates of Sclerotinia homoeocarpa

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DMI insensitivity or resistance has become widespread in populations of Sclerotinia homoeocarpa, the causal agent of dollar spot in turfgrasses. The induced expression of ShCYP51 as influenced by the length of DMI exposure was quantified in six sensitive and six insensitive isolates. ShCYP51 expression increased in all isolates after exposure to propiconazole at 0.5 μg/ml for 1 h, 24 h or 48 h when compared to the constitutive level of expression. However, 24 h and 48 h exposures resulted in significantly higher levels of expression in the insensitive group than in the sensitive group. In addition, expression of the gene was greater at 48 h than at 24 h in the insensitive isolates. No corresponding expression was observed in the sensitive group. Comparing expression of ShCYP51 among individual isolates, no differences were detected in constitutive expression or after 1 h propiconazole exposure. Expression was greatly increased for all isolates after 24 h of exposure, but after 48 h of propiconazole exposure, five of six insensitive isolates exhibited higher levels of expression than six sensitive isolates. Results from this study show that expression of ShCYP51 following DMI exposure is an important factor determining DMI sensitivity in S. homoeocarpa. In addition, the findings that the level of ShCYP51 expression was positively correlated with the duration of DMI exposure suggests that increased rate or frequency of DMI fungicide application could magnify ShCYP51 expression levels and further reduce disease control.

Classification of strains of Xylella fastidiosa isolated from pecan in Louisiana as Xylella fastidiosa subspecies multiplex

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Xylella fastidiosa causes disease in a number of economically important crops and landscape trees including grapevine, citrus, coffee, peach, oleander, oak, and sycamore. In pecan, X. fastidiosa causes pecan bacterial leaf scorch (PBLs) disease, which causes defoliation and reduces nut yield. Even though X. fastidiosa infection is chronic, no economically effective treatment methods are available. While some level of host specificity exists within this bacterial species, the basis for this specificity is not yet clear. In order to develop better management practices for PBLs, it is necessary to identify the subspecies of the pathogen that infect pecan so that potential differences in host specificity in the pathogen might be elucidated.

Response of Meloidogyne incognita to silicon

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The mechanism of Silicon increasing resistance in crops is poorly understood and the effect of Silicon on plant-parasitic nematodes is unknown. Silicon-mediated resistance has been observed in coffee and cucumber to Pythium ultimum and Meloidogyne exigua; however, no study has investigated the effect of Silicon on Meloidogyne incognita, an important pathogen on cucumber. Thus, the objectives of this study were to determine if applying Silicon as a soil application increased resistance in cucumber to M. incognita and evaluate the response of M. incognita to Silicon. In a greenhouse study, 364.4 μg/ml (Kasal 6 26.6% SiO2) applied as a root dip reduced (P = 0.05) nematode reproduction compared to applying Silicon near roots in soil. Inoculated plants from a nematode density assay the LDI of M. incognita at 24 h after exposure was 6.786 μg/ml of SiO2, which was higher than anticipated. Thus, the sensitivity of M. incognita to Silicon used in this study may have contributed to lower nematode reproduction on roots dipped in Silicon. Further studies are underway to completely understand the mechanism of Silicon increasing resistance in cucumber to M. incognita and effect of Silicon on the pathogenicity of M. incognita.

Impact and management of root-knot nematode on soybean in Arkansas

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Two trials were conducted to determine efficacy (and practicality) of applying currently labeled and potential nematicides for controlling root-knot nematode (Meloidogyne incognita) in soybean. The impact of combining nematicide treatments with currently available resistant cultivars was also evaluated. Both trials were located in Craighead County, Arkansas near Black Oak. Various labeled and un-labeled nematicides, including Telone II, Temik, Vydane (folar and infurrow), seed treatments – Aerts, Avista, and Vydane were evaluated. Plant damage (root galling and vigor) and yield were evaluated. Nematode pressure was moderate to severe at both locations resulting in measurable plant damage and yield loss. Root galling damage ranged from 3 to 9 (where 0 = no galling and 10 = 75% or more of the root system galled) with Telone having the lowest level of damage compared to the untreated control in the susceptible variety. Vydane applications in-furrow and foliar also suppressed root-knot damage compared to the untreated control. The moderately resistant variety did not respond significantly to the application of a nematicide, whereas the susceptible variety showed a significant yield response to both Vydane and Telone treatments. Seed treatment nematicides did not perform at the level of Telone or Vydane in controlling root-knot nematode in the susceptible variety. The moderately resistant variety performed effectively in reducing nematode damage and maintaining yield potential without a nematicide.

Implications of strobilurin fungicide-resistant Cercospora sojina in Tennessee

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Frogeye leaf spot (FLS) of soybean, caused by the fungus Cercospora sojina, was found to be highly resistant to strobilurin fungicides in leaf samples collected from a soybean production field in Lauderdale County, Tennessee in 2010. Frogeye leaf spot is the number one foliar disease in Tennessee, causing an average yield loss of 7.8% statewide. Many producers spray one to two applications of strobilurin fungicides to reduce the yield loss from FLS and other foliar diseases, especially when crop rotation or resistant varieties are not utilized. In laboratory tests conducted at the University of Illinois, spores from the Tennessee isolates of C. sojina were found to germinate in the presence of high concentrations of selected strobilurin fungicides. Currently, this is the only known report of strobilurin-resistant C. sojina in the United States. Soybean growers are urged to manage FLS through the use of resistant or tolerant varieties, crop rotation and use of effective triazole or triazole-strobilurin fungicides when susceptible varieties are used. Research recently conducted in Tennessee has shown that a number of varieties are resistant or tolerant to FLS. Foliar fungicide tests have shown good control of FLS with some triazole fungicides on susceptible varieties.

Validation of a pecan scab prediction model using repeated measures logistic regression analysis

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Fusarium oxysporum (syn. Cladosporium caryigenum), causal agent of pecan scab, is the most economically destructive fungal pathogen of pecans (Carya illinoinensis). Severe epidemics of pecan scab can reduce crop yield and quality. To manage pecan scab, fungicides sprays are routinely used. A weather-based advisory currently used to assess fungicide application requires the accumulation of scab hours (SH). SH is defined as an hour of average temperature above 7°C and 90% relative humidity. Disease severity (DS) was assessed on fruit multiple times per season from 1994–1996 and 2009–2010. Weather parameters examined were T, RH, total solar radiation (SR), and total rainfall (R) collected daily from the Oklahoma Mesonet weather stations. R and DS were converted to binomial variables where a rain event (R ≥ 2.5 mm) and disease severity (DS ≥ 25%) was coded as 1 and all other events as 0. Logistic models were developed using generalized estimating equations. The best fitting model included all main effects where DS = −33.40 + 0.1803 RH + 0.3806 T − 0.5770 SR + 3.39 R (QICu = 313.31). Under no-rain and SR = 22.5 MJ m−2 the probability of economically damaging levels of pecan scab occurring when T = 21°C and RH = 90% is 0.62. This model validates previously established SH thresholds, but also incorporates the biologically important influences of solar radiation and rain in scab development.

Epidemiological studies on Blackberry yellow vein associated virus

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Blackberry cultivation, especially in the Southeastern United States is flourishing along with the increasing consumer demand and release of new cultivars suitable for fresh market. However, Blackberry yellow vein disease (BYVd), a disorder caused by virus complexes has become a major threat for blackberry production in the area. Blackberry yellow vein associated virus (BYVav), a recently identified crinivirus is the most prevalent virus in the BYVd complex being detected in over 50% of sample exhibiting BYVd symptoms. The virus is asymptomatic in single infection and acts synergistically during co-infection with other viruses to cause disease. The objective of this study was to garner more knowledge on BYVav that includes identification of initial sources of infection, vector(s) and alternative hosts. Several isolates of the virus infecting cultivated and wild blackberries were collected from different states with high BYVd incidence for the diversity study. The variability was determined after cloning and sequence analysis of four different genomic regions of the virus; the regions being the most genetically diverse among viruses in the family Closteroviridae. Twenty-seven plant species from blackberry fields with high BYVav incidence were collected and tested as possible alternative hosts. Whiteflies are known vectors of criniviruses and -thus the greenhouse whitefly was tested for its ability to transmit the virus. The results of this study clarify factors contributing to the epidemiology of BYVav, the primary component of the emerging BYVd by identifying the putative geographical origin of the BYVav, its potential vectors and alternate hosts.

Morphological characterization and fungicide sensitivity of Phytophthora species in Texas

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Phytophthora blight in vegetable production in Texas has been associated with the oomycete Phytophthora capsici. Six isolates of Phytophthora sp. were obtained in 2008 from diseased pumpkin fruit (Cucurbita pepo) in Yoakum County, Northwest Texas, and two isolates were obtained from diseased watermelon fruit (Citrus lananaus) in Hidalgo County, South Texas. Both locations had P. capsici in recent years. These isolates were characterized morphologically, and assessed for fungicide sensitivity to mefenoxam and mandipropamid. One isolate from pumpkin had obpyriform and non-papillate sporangia, which is atypical for P. capsici observed in Texas. This isolate was P. capsici isolate 1.7 (1) which is similar to breadth ratios B, respectively sporangia, which is typical for this species. One isolate from watermelon averaged 1.8, while the other failed to produce sporangia. The sporangia of five isolates from pumpkin varied from ellipsoidal to spherical, although sporangial production was poor in some cases. Only one isolate from pumpkin was determined to be intermediate in resistance to mefenoxam. All remaining isolates from pumpkin and watermelon were sensitive. All the isolates were highly resistant to high concentrations of mandipropamid. Based on morphological characterization, all isolates seem to fit with typical parameters for P. capsici except for one isolate from pumpkin. None of the isolates were found to be resistant to either mefenoxam or mandipropamid. The potential may exist for atypical populations of P. capsici to be present or for another species associated with fruit rot. Although sensitivity to mefenoxam has decreased in isolate populations over time, resistance to mefenoxam has yet to be detected in Texas.

Chemical control of Cercospora leaf blight of soybean: Evaluation of fungicide efficacy and time of application

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Cercospora leaf blight of soybean (CLB), caused by Cercospora kahakii, is a major disease of soybeans in Louisiana and the Gulf South. Previous studies demonstrated varietal tolerance and resistance, but resistance was overcome within two years. Varieties that did well in one location showed considerable differences in other parts of Louisiana. Timing of disease onset is a key factor in crop impact, which can result in substantial reductions in yield and soybean quality. The purpose of this study was to evaluate stroblurin, triazole and chlorothalonil fungicides for disease control and to evaluate time of application of this as may relate to time of infection. Several application protocols were assessed including first applications at V5 continuing through R6 with no more than two applications per treatment. Maximum label rates were applied. Plots were rated for disease severity at mid-R6. Our findings indicate an early application, probably at late vegetative stages, will be required. Additionally, two applications may be required for optimal disease control. There is some indication that stroblurins are not as effective as triazoles, which confirms earlier work.

Expression of NAC-like transcription factor is involved in bacterial panicle blight resistance in rice

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The NAC transcription factors are involved in plant growth and development, and play roles in plant responses to both abiotic and biotic stresses. Microarray data showed that a NAC-like transcription factor (NTF) in rice is up-regulated after inoculation of rice with the bacterial pathogen, Burkholderia glumae. The major causal agent of bacterial panicle blight (BPR) in rice. In order to determine if the NTF is involved in resistance to B. glumae, the expression of the NTF was studied in a partially resistant rice cultivar, Jupiter, and a susceptible rice cultivar, Trennase. Two of these cultivars were inoculated at 30% rice heading initiation with 1 x 10 8 cfu/ml of a virulent strain of B. glumae. 336gr-1, a tox derivative of 336gr-1 lacking toxoflavin production, and a tox hrp derivative of 336gr-1 lacking toxoflavin production and a functional type III secretion system. Total RNA were extracted and reverse-transcribed from rice panicles collected at 24 h post inoculation for each inoculum treatment. Gel electrophoresis of the reverse transcription products showed that the NTF was induced in Jupiter inoculated with 336gr-1, 336gr-1 tox and 336gr-1 tox hrp. In contrast, induction of the NTF gene was not observed in Trennase treated with the same bacteria. These results suggest that expression of the NTF gene is involved in resistance to B. glumae in rice. NTF gene expression in Jupiter panicles inoculated with 336gr-1 tox or 336gr-1 tox hrp was induced more than the panicles inoculated with 336gr-1. This indicates that toxoflavin and the TSSM may suppress the NTF gene expression in the partially resistant variety. We are currently quantifying NTF gene expression with real time PCR and performing complementation assays to verify these preliminary results.

Field validation and in vitro confirmation of temperature and relative humidity variables used to predict fungicide application for the control of dollar spot of creeping bentgrass

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Dollar spot, caused by Sclerotinia homoeocarpa, is the most damaging disease of closely mown creeping bentgrass and annual bluegrass putting greens. Repeated fungicide applications are typically required to control dollar spot and maintain acceptable turfgrass quality. To improve efficiency of fungicide programs disease prediction models are often used. Previously, logistic regression was used to develop a model that input weather variables to predict probability of dollar spot development (PY). The model inputs include 5-day moving averages of minimum air temperature [MNT], average relative humidity [RH], and a class variable for fungicide [FUNG] use [1 = fungicide used; 0 = no fungicide used] to describe PY. According to the model, 5-day average MNT above 14°C was conducive for the development of dollar spot. When 5-day average MNT was between 14°C and 23°C, 5-day average RH of 75% or above was considered sufficient for dollar spot development. The MNT and RH thresholds were confirmed using two controlled environment chamber experiments where: 1) growth of S. homoeocarpa was monitored on
the surface of soil amended with grass clippings; 2) dollar spot symptom development was monitored in small pots containing creeping bentgrass. In 2010, the predictive model was used to time fungicide applications in independent field validation studies in OK and WI. Compared to 14-day calendar-based program, the model required six fewer fungicide applications in OK and correctly identified periods of weather that were conducive for the disease. In WI, no reduction in fungicide applications occurred using the model; however, periods conducive for dollar spot during the season were correctly identified. These results suggest that the model correctly identified weather conditions that favor dollar spot, even in widely varying environments.

**Relationship between frequency of fungicide resistance and efficacy in the management of gummy stem blight of watermelon**

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Gummy stem blight (GSB), caused by the fungus Didymella bryoniae, is the most destructive disease of watermelon and is primarily managed with fungicides. *D. bryoniae* has developed resistance to many fungicides that were once very effective, including azoxystrobin, boscalid and thiophanate–methyl. Field experiments were conducted in Tifton (TN) and Reidville (RV), GA in 2009 and 2010 to establish a relationship between frequency of fungicide resistance based on in vitro assays and fungicide efficacy. In 2010, in both locations, the frequency of resistance to boscalid, thiophanate-methyl and azoxystrobin was ≥0.90 in isolates collected from non-treated plots. All isolates collected after six applications of boscalid, thiophanate-methyl or azoxystrobin were resistant to each fungicide. All isolates collected from the non-treated plots were sensitive to tebuconazole. Isolates from tebuconazole-treated plots in one of the locations showed slightly reduced sensitivity to tebuconazole, but did not result in control failure. GSB severity was assessed at 63 and 70 days after planting in TN and RV, respectively. GSB severity in plots treated with boscalid, thiophanate-methyl or azoxystrobin was not significantly different from the non-treated plots (45%, TN and 16%, RV). GSB severity in tebuconazole-treated plots (14%, TN and 4%, RV) was significantly lower than the non-treated control. There was a consistent negative association between frequency of fungicide resistance and disease control in the field. Thus, knowledge of the frequency of fungicide resistance in the pathogen population will be helpful in selecting the most effective fungicides for the management of GSB in watermelon fields.

**Evaluation of reduced aegctard rates and frequency on bacterial spot management in Florida**

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In Florida, *Xanthomonas perforans* is the principal cause of bacterial spot on tomato. Control has relied almost exclusively on copper bactericides mixed with mancozeb; however, due to the prevalence of copper resistance control is marginal. Azoxystrobin (ASM), the active ingredient of Aegctard (Syngenta, Greensboro, NC), is a chemical elicit that has demonstrated efficacy against several diseases including bacterial spot on tomato. However, the adoption of ASM by many growers has been less than enthusiastic over concerns of reduced plant vigor and yields; which have occasionally been documented to cause yield losses of over 80% in Africa, South America, and the southern US and up to 100% in Asia. In the southern US, the fungus overwinters on kudzu. Despite the presence of inoculum in early spring, rust on soybean usually does not occur until late in the summer. Preventative chemical fungicides are often applied during reproductive stages (R1-R3), but results can be inconsistent and applications are costly. In order to examine the latent period of SBR, we used quantitative real-time PCR (qPCR) to detect early infections of *P. pachyrhizi* on soybeans in Louisiana and Florida. In 2009, sporulating SBR was documented in February on kudzu in Baton Rouge, LA. Soybeans planted in April developed SBR symptoms during the R5 growth stage. However, we detected latent infection while soybeans were in the mid-reproductive stages, more than 60 days before symptom development. qPCR analysis indicated similar results for adjacent fields planted in May, June, and July with latent periods ranging from 20 to 60 days, and symptoms developed after seed set. In 2010, following several freezes and complete dieback of kudzu, SBR was detected in Quincy, FL on kudzu in mid-July and on soybeans at R6 that had been planted in July. Results from qPCR assays showed infection as early as flowering, approximately 20 days earlier than symptom development. Throughout this study, latent periods varied from 20 to 60 days. We conclude that symptom development is a function of plant growth stage, regardless of time of infection. Therefore, it may be more efficacious to commence fungicide applications at the time of initial infection rather than at specific plant growth stages.

**Effects of mineral nutrients on Cercospora kikuchii and Cercospora leaf blight in soybean**

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Phytopathology 101:S269

Cercospora leaf blight (CLB) has become the most important disease of soybean in Louisiana. The disease is variable from year to year and can cause disease losses of 15 to 30%. Epidemics typically begin after seed set with defoliation occurring in severe cases. The pathogen, *Cercospora kikuchii*, produces a disease complex which also includes purple seed stain. The seedborne fungus serves as an initial inoculum source for subsequent infections. Protocols are not yet established for effective chemical control, and these applications are costly. In other host-parasite systems, it is known that plants deficient in certain elements are more susceptible to disease. This may also apply to CLB. Therefore, we evaluated the mineral nutrients boron, manganese, zinc, copper, and iron. Laboratory experiments included quantification of mycelial growth on solid and liquid substrates as well as quantification of cercospordin production. Additionally, foliar treatments were applied to field grown soybeans at seed set. These findings indicated that iron significantly inhibited growth of *C. kikuchii* colony diameter up to 3-fold after 14 days and a 10-fold reduction in mycelial biomass in liquid cultures at 28°C. Therefore, we evaluated the disease severity in field trials following foliar applications of iron, even at low doses. Other minerals resulted in no significant reductions in disease. Our results suggest that iron may be effective in the management of CLB, either alone or in combination with other disease control strategies.

**Detection and identification of the fungus Aspergillus flavus in maize using solid-phase microextraction**

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Phytopathology 101:S269

*Aspergillus flavus* accumulates in maize during pre- and postharvest, and the detection of toxigenic isolates of the fungus is important to ensure food safety. To date, most methods used for detection are destructive which require harvesting the ears. This study describes a potential non-destructive method utilizing volatile organic compounds (VOC's) in maize. The objective of this project is to rapidly detect *A. flavus*-infested vegetative and reproductive structures in maize by utilizing headspace solid-phase microextraction (HS-SPME) and a portable mini gas-chromatograph (GC). Replicated experiments were performed in the laboratory and field employing the heavy aflatoxin-producing strain, NRRL 3357 for inoculations. A 65-microgram Polydimethylsiloxane/Divinylbenezene SPME fiber was determined in preliminary experiments to be the optimum fiber for evaluating the sample preparation technique. A unique volatile profile was assembled by GC-Mass Spectrometry (GC-MS) for all samples measured. To identify HS-VOC's unique to toxigenic strains of *A. flavus*, strain NRRL 3357 was grown on sterilized 2% corn meal broth and incubated for 1 week at 37°C. HS-VOC's were collected and desorbed every 24 hours for 1 week. Sterilized corn meal broth was used for comparison in all laboratory studies. For field experiments, NRRL 3357 was used for inoculation of maize ears 65 days after tasseling; HS-VOC's were collected once a week for 4 weeks. Non-inoculated maize ears were used for comparison in all field experiments. A total of 34
Life after aldicarb: Management of the root-knot nematode-Fusarium wilt disease complex in cotton

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Phytopathology 101:S270

The root-knot nematode-Fusarium wilt disease complex (caused by Meloidogyne incognita (M) and Fusarium oxysporum f. sp. vasinfectum (Fov)) is an economically important disease of cotton (Gossypium hirsutum). Historically, aldicarb has been the commercial standard for management of this disease on the southern High Plains of Texas; however, the manufacturer (Bayer CropScience) will cease production. Extensive research has been conducted to evaluate alternative management strategies for Fov and Mi. Results of field trials have shown that use of partially resistant cultivars can improve yields 1037 to 2189 kg/ha over susceptible commercial standards in fields co-infested with Fov and Mi. Additional studies indicate that rotation with a non-host, such as peanut (Arachis hypogaea) can adversely affect Mi populations resulting in less damage to cotton roots the subsequent year. Furthermore, the use of fumigants, such as 1,3-dichloropropene, have led to increases in profitability ($59-151 per ha) in fields infested with Mi. Discontinued production of aldicarb in 2014 will greatly impact cotton production in fields infested with Mi and/or Fov, thus an increased emphasis will be placed on identifying effective nematode management strategies.

Potyvirus and insect vector movement in Louisiana sweetpotato fields

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The potyviruses, Sweet potato feathery mottle virus (SPFMV), Sweet potato virus G (SPVG), and Sweet potato virus 2 (SPV-2), frequently infect sweetpotato in Louisiana, often as mixed infections, but little is known about population dynamics of their aphid vectors. To study vector populations, aphids were trapped in and around sweetpotato crops on a weekly basis using green and yellow pan traps and yellow sticky traps in plots at the Burden Center in Baton Rouge, and the Sweet Potato Research Station in Chase, and in three commercial farms in St. Landry, West Carroll and Morehouse Parishes, Louisiana from March through September in 2009 and 2010. Ipomoea setosa virus sentinel plants were placed in fields for one week and then transplanted to a greenhouse for two more weeks to monitor virus transmission. Plants showing symptoms were tested for SPFMV, SPVG, and SPV-2 using NCM-ELISA. Although aphids were captured during the entire crop cycle, virus infection of sentinel plants occurred mainly in the fields during the months of June/July in 2009 and July–September in 2010 with very little transmission in plant beds. NCM-ELISA tests on symptomatic sentinel plants for SPFMV were positive, with prevalence ranging from 89-100% at all locations, while SPVG and SPV-2 were detected in some fields at low incidence (5-28%). The most common aphid species captured in pan traps were Rhopalosiphum padi, Aphis gossypii and Macrosiphum euphorbiae. The most predominant aphid in 2009 was R. padi in West Carroll (44%) and Chase (48%), M. euphorbiae (28%) in St. Landry and A. gossypii (32%) at Burden. Although vectors were present throughout the entire sweetpotato growing season, virus infection did not occur in a restricted period suggesting inoculum may be a limiting factor.

Genetic diversity among Sclerotium rolfsii isolates in southeast U.S.

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Southern blight (caused by Sclerotium rolfsii Sacc.) is a serious fungal disease affecting crops grown around the world, especially in tropical and subtropical regions. The disease is becoming more problematic in vegetable production in the southern United States with phase out of methyl bromide and the adoption of organic and other low-input production strategies. Sixty-three isolates from several hosts, including peanut, pepper, and tomato were partially characterized for mycelial compatibility and pathogenicity. The 63 isolates were assigned to 19 mycelial compatible groups (MCGs), of which 11 MCGs were exclusive to single hosts. However, six MCGs consisted of isolates originating from several hosts. No common MCGs were observed among peanut and vegetable isolates, with the exception of a single peanut isolate. A single representative isolate from each MCG was chosen for pathogenicity tests on potato, pepper, and tomato. All isolates were pathogenic on all hosts, but significant differences in virulence were observed among isolates on peanut and pepper. As a group, isolates from peanut were more virulent on peanut and pepper than isolates from other hosts. No significant difference in virulence was detected among S. rolfsii isolates on tomato, but additional tests using reduced levels of inoculum are necessary. While considerable genetic diversity exists among S. rolfsii isolates, results suggest some level of host adaptation, especially among peanut isolates. Further characterization of these and additional isolates is in progress.

A new virus disease in the U.S.: Soybean vein necrosis

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Phytopathology 101:S270

A new virus disease was discovered in soybean. Affected plants exhibit vein-clearing in early growth stages, followed by necrosis. The disease has been found in high incidence in all areas surveyed and symptoms seem to be genotype-dependent with some cultivars exhibiting only vein clearing and others showing extended areas of necrosis. The causal agent is a new tospovirus provisionally named Soybean vein necrosis virus (SVNV). The tripartite genome has been completely sequenced and the three genomic molecules encode five open reading frames and six proteins. Phylogenetic analysis based on the polymerase, clearly place SVNV in a unique place, sharing similar phylogenetic distances from the TSWV and INSV/MYGV isolates but SVNV a unique evolutionary link in the genus. This hypothesis is also supported by its minimal similarity with other tospoviruses. Over 35 isolates were collected from seven states to study virus diversity and develop reliable molecular tests, able to detect all isolates in the field. In contrast to the phylogenetic analysis which indicates that SVNV is an ancient virus, the diversity analysis concluded that the virus has recently emerged in soybean as all isolates were very closely related. Identification of the thrips vectors and alternative hosts for the virus are underway. The results of this study will provide further insight into the virus evolution and hopefully identification of the original host of SVNV.

Management of bacterial panicle blight of rice with beneficial Bacillus subtilis Zk-0

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Phytopathology 101:S270

Bacterial panicle blight, caused by Burkholderia glumae, poses an increasing threat to rice production in the southern United States as well as in other rice-growing areas in Central and South America and Asia. The disease was widespread in Texas, Arkansas and Louisiana during the 2010 crop season. Currently, there are no rice cultivars with high levels of resistance to this disease and registered chemicals to use in the U. S. In this study, plant growth-promoting rhizobacteria (PGPR) were evaluated for suppression of bacterial panicle blight in rice. Among 17 strains of PGPR evaluated, seven strains showed significant increase in yield by 11 to 17%. Mixture of these two strains or combined use of either strain was more effective (more than 57%) in reducing severity of bacterial panicle blight. These seven strains were then tested in the greenhouse by spraying them onto flowering panicles of rice challenged with B. glumae. Among these seven strains, two strains, belonging to Bacillus subtilis, were most effective (more than 57%) in reducing severity of bacterial panicle blight. These two strains were further evaluated under artificially-inoculated field conditions in Texas by spraying them on rice panicles at the flowering stage. The results showed that these two strains reduced disease severity by 41 to 50% and increased yield by 11 to 17%. Mixture of these two strains or combined use of either strain with oxalic acid did not further increase yield compared with the strains alone. Use of PGPR strains may provide practical solutions to minimize the damage caused by bacterial panicle blight of rice.

Disease severity and yield potential of rice cultivars in organic production systems

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Phytopathology 101:S270

The market demand for organically produced rice has driven the steady increase in acreage of organic rice in the U. S., with Texas and California having the most acreage. Yield potential and disease management are among the principal challenges associated with organic rice production. We evaluated unique compounds were detected in the toxigenic strain and assembled production in fields infested with Mi and/or Fov, thus an increased emphasis will be placed on identifying effective nematode management strategies.
27 rice cultivars and breeding lines to determine their responses to diseases and yield potential under organic production conditions in Texas over two years. Narrow brown leaf spot (*Cercospora janseana*) was a constant yield-limiting disease and was severe on *Jazzman*, *Presidio*, *Sierra*, *Cocodrie* and the lines derived from *Cocodrie* in both tilled, water seeded and no-tilled, dry seeded production systems. In the no-tilled production system with a significant amount of previous cover crop residue, straighthead was another significant disease, occurring in all the cultivars and lines, with *Cocodrie* and its derived lines having the most severe symptoms. Brown spot (*Cochliobolus miyabeanus*) was also commonly present regardless of the production system used whereas sheath blight (*Rhizoctonia solani*), bacterial panicle blight (*Burkholderia glumae*), and leaf smut (*Entyloma oryzae*) were minor in severity for the majority of the cultivars and lines. The cultivars *Tesanai 2*, *GP2*, *Rondo*, PI312777, and PI338046 had the lowest levels of all these diseases and produced yields that ranked among the highest. *Tesanai 2* outyielded all other cultivars and lines. These results can help identify rice cultivars suitable for organic production relative to disease susceptibility and yield potential.
2011 Potomac Division Meeting Abstracts

The abstracts presented at the APS Potomac Division meeting in Rehoboth Beach, Delaware, March 9–11, 2011. The abstracts are arranged alphabetically, by first author’s name.

Statistical tools useful in characterizing the molecular response of cacao to *Moniliophthora roreri* infection
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M. *Moniliophthora roreri* (Mr) causes frosty pod rot, a destructive disease on *Theobroma cacao* (cacao). Pods were inoculated with Mr spores in the field and assessed for disease. Total RNA was extracted from pods harvested 7, 30, 60, and 90 days after inoculation and QPCR analysis was carried out using primer sets for 5 Mr ESTs and 89 cacao ESTs. Disease symptoms and Mr EST expression levels in infected pods increased rapidly between 7 and 60 DPI. ANOVA consistently identified individual cacao ESTs responsive to development and infection but was dependent on fixed inoculations and harvest times. Multiple regression analysis allowed fixed inoculation and harvest times to be replaced with independent variables such as pod size and fungal gene expression but still only provided information on individual cacao ESTs. The cacao expression data were analyzed across experiments and ESTs using principle coordinates analysis (PCoA) techniques. PCoA allowed consideration of how individual cacao EST expression was altered in response to Mr infection and development relative to all the ESTs studied in a single analysis. A Mantel test of data from 2 independent experiments indicates that the molecular signature for the susceptible response to Mr is highly reproducible. These techniques should allow us to expand our understanding of the Mr/cacao interaction using field samples without fixed treatments, incorporating divergent plant and pathogen genetic backgrounds.

Leaf anthracnose, a new disease of swallow-worts from Russia
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Phytopathology 101:S272

Black swallow-wort (*Vinceptoxicum nigrum* (L.) Moench (=*Cynanchum louiseae* Kartesz & Gandhi) and pale swallow-wort (*Vinceptoxicum rossicum* (Kleopow) Borhidi (=*Cynanchum rossicum* (Kleopow) Borhidi) are invasive plants belonging to the family Apocynaceae and are the targets of biological control efforts to control their spread in the U.S.A. In 2010, diseased leaves of a related species, *V. scandens* Sommier & Levier, were collected in the Krasnodar area of Russia and sent to the BLS-3 containment facility at the USDA-ARS-FDWSRU for further investigation. Seeds of *V. scandens*, collected simultaneously in Russia, were placed in a freezer at –20°C for 6 weeks and then germinated in sterile Petri plates on moist filter paper. The seedlings were then transplanted and grown in a 20°C greenhouse under 12 hours of light. Five two-month-old plants each of *V. scandens*, *V. nigrum*, and *V. rossicum* were inoculated with spores from two-week-old cultures of isolate 10-002. Plants were inoculated by spraying an aqueous suspension of 10⁶ spores per ml onto each plant until all leaves were wet. The plants were placed in 20–24°C dew chambers for 18 hours and then placed in a 20°C greenhouse. Two weeks later, diseased leaves of each species were harvested, and the fungus was re-isolated from each species. Spore and appressoria measurements conformed to the description of *Colletotrichum lineola* Corda, and ITS sequences of this isolate (GenBank # HQ731491) aligned 100% to 15 isolates of *C. lineola* in GenBank. Voucher specimens of the fungus have been deposited in the U.S. national fungus collection.

Previously undescribed phytoplasmas in diseased plants of passion fruit (*Passiflora edulis* f. *flavicarpa* Deg.)
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Phytopathology 101:S272

Diseases of passion fruit (*Passiflora edulis* f. *flavicarpa*, family *Passifloraceae*) possibly associated with infections by phytoplasmas have been observed in Brazil, where the plant is widely cultivated and used in food products and beverages. Although the association of phytoplasmas with passion fruit disease has been recognized for over 30 years, little work has been done to characterize the phytoplasmas. In the present study, passion fruit plants exhibiting symptoms of witches’ broom growths in Brazil were investigated for possible infection by phytoplasma. Analysis of 16S ribosomal (r) DNA amplified in the polymerase chain reaction (PCR) indicated that the symptomatic plants were infected by two distinctly different phytoplasma strains. Based on results from actual and virtual (*PhyClassifier*) RFLP analysis of 16S rDNA, one strain (**PassWB-Br3**) represents a previously undescribed subgroup lineage in group 16SrIII (X-disease phytoplasma group); the other strain (**PassWB-Br3**) represents a previously undescribed subgroup lineage in group 16SrVI (clover proliferation group, ‘Candidatus Phytoplasma trifolii’-related strains). Phylogenetic analyses and nucleotide sequence alignments of 16S rRNA gene sequences revealed that strain **PassWB-Br3** differed from all previously described ‘Ca. Phytoplasma’ species and should be recognized as representative of a new ‘Ca. Phytoplasma’ species.

An investigation into heomeostasis of gibberellin in potato purple top phytoplasma-infected tomato plants
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Phytopathology 101:S272

Phytoplasmas are a group of phloem-restricted, cell wall-less bacteria responsible for numerous plant diseases. Plants infected by phytoplasmas exhibit various symptoms believed to be the results of hormonal imbalance. Gibberellins (GAs) are essential phytohormones that regulate growth and...
development of plants. It has been shown that exogenous application of GA can effectively remit dwarf symptoms caused by phytoplasma and spiroplasma infections, a strong indication of the shortage of endogenous GA in mollicute-infected plants. The purpose of the current study was to investigate the phytoplasma-induced changes in expression profiles of GA metabolism genes, and to examine whether exogenous GA application would restore GA homeostasis in potato plants. Potato plants and Rutgers tomato were used as a model pathogen-host pair in this study. Results revealed that, compared with healthy (mock-inoculated) plants, transcript levels of two GA biosynthesis genes, GA20ox2 (encoding GA 20-oxidase 2) and GA3ox2 (encoding GA 3-oxidase 2), were notably decreased in PPT-infectected plants. Transcript levels of a GA catabolism gene, GA2ox1 (encoding GA 2-oxidase 1), were also slightly reduced in PPT phytoplasma infected plants. Exogenous application of gibberellic acid (GA3) significantly attenuated the “big bud” symptoms on PPT-infected plants. Expressions of GA20ox2 and GA3ox2 were significantly down-regulated after GA application in PPT-infected plants. On the other hand, expression of GA2ox1 was dramatically up-regulated upon GA treatment. These results indicated that i) GA homeostasis was maintained by feedback regulation of GA metabolism genes; ii) phytoplasma infection caused a shift in GA homeostasis; and iii) exogenous application of GA3 was able to partially reverse the shift, toward normal GA homeostasis. Findings from the present study will aid our understanding of the role of GA in phytoplasma pathogenesis and exogenous GA-induced phytoplasmal disease symptom remission.

Resting spores for long-term storage of Synchytrium solstitiale, a candidate for biological control of yellow starthistle

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Phytopathology 101:S273

An isolate of Synchytrium solstitiale from California has been recently found for biological control of yellow starthistle (YST, Centaurea solstitialis). Protocol was needed for long-term storage of S. solstitiale for research and archival purposes. In greenhouse studies, germination of mature resting spores of S. solstitiale resulted after storage in dried YST leaves for >2.5 years. Dried YST leaves were surface sterilized, and resting spores galls were removed by scraping or grinding leaf tissue. Spores were placed on 2% water agar in Petri dishes that were wrapped with Parafilm and aluminum foil and incubated at 10/15°C (night/day temperatures). One vesicle per resting spore, each with a single sporangium (= sorus), developed in 7–20 days. Zoospores were released from sori in sterile distilled water with 100 ppm Streptomycin. Plants also inoculated with sori from resting spores were incubated in moist plastic bags at 10/15°C (night/day temperatures) and an 8-hour photoperiod. Plants were removed from the growth chamber after 10 days, placed in a 20°C greenhouse, and observed for symptom development. Successful germination and plant infection occurred from inoculation by resting spore galls following this protocol. A test to measure viability and virulence of resting spores after 1.4 or 2.6 years of storage resulted in successful germination and infection of YST plants using the protocol described. Thus, long-term storage and maintenance protocol for S. solstitiale has been achieved.

The rice blast fungus, Magnaporthe oryzae, copes with plant-generated reactive oxygen species through the virulence factor MohYR1

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Phytopathology 101:S273

During plant-pathogen interactions, the plant may mount several types of defense responses to either block the pathogen completely or ameliorate the amount of disease. One such response is the production of reactive oxygen species (ROS). On the other hand, a successful pathogen will likely have its own ROS detoxification mechanisms to cope with this inhospitable host species (ROS). One such response is the production of reactive oxygen species through the virulence factor MohYR1. MohYR1 mutants became less sensitive to exogenously applied H2O2. Together, our data suggest that HYR1 is a virulence factor in the rice blast pathogen, and its role in virulence is directly related to sensing and managing ROS generated during early infection events. We are also working on understanding how HYR1 and YAP1 interact to manage ROS, and data will be presented on initial interactions.

Plant hormones as potential biomarkers of early phytoplasma infection

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Phytopathology 101:S273

Phytoplasmas, a diverse group of unculturable cell wall-less bacteria, are causative agents of numerous plant diseases affecting a broad range of agriculturally and environmentally important plant species worldwide. Phytoplasma-infected plants often exhibit symptoms suggestive of a disturbed hormonal balance. The current study was designed to investigate changes in the endogenous levels of four major plant hormones in infected plants, and to determine whether plant hormones can be exploited as biomarkers of phytoplasma infection. Columbia Basin potato purple top (PPT) phytoplasma (a member of subgroup 16SrVI-A) and its alternate host Rutgers tomato were used as a model pathogen-host pair in our study. Paraflin-embedded tissue sections prepared from various parts of PPT phytoplasma-infected and healthy tomato plants were subjected to a semi-quantitative immunohistochemical assay optimized in our laboratory. Results from the study revealed that, in PPT-infected plants, levels of abscisic acid (ABA) and cytokinins (N6-benzyladenosine, trans-zeatin riboside, and cis-zeatin riboside) were elevated, and the levels of auxin (IAA) and gibberellic acid (GA3) were decreased. A time-course analysis indicated that changes in hormonal level were most evident in the stem, with data collected at 10 days post graft-inoculation, a time point when no disease symptom was visible and no PPT phytoplasma DNA was detectable using nested polymerase chain reactions. Our findings suggest that endogenous levels and ratios of plant hormones are potential pathological markers of early phytoplasma infection. Findings from the study will aid our understanding of the role of plant hormones in phytoplasma pathogenesis and disease symptom expression.

A novel way to prepare plant DNA using the GenomiPhi DNA Amplification Kit

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Phytopathology 101:S273

Plant species vary widely in their genome size and tissue composition. The tissues of many plants contain high levels of polyphenols and polysaccharides which can interfere with DNA extraction. Polyphenolic and polysaccharide substances can still interfere with restriction enzymes and DNA polymerases which can make the DNA unusable for molecular analysis and research applications. The GenomiPhi™ DNA Amplification Kit has been successfully validated to amplify extracted DNA from a variety of plant seeds and leaves. We present data that shows the generation of microgram amounts of DNA from small amounts of input DNA. Using a simple extraction method, such as alkaline lysis, or a more detailed method, such as the CTAB method, the amplification of plant DNA was equally successful using the GenomiPhi DNA amplification method.

Gene expression profiling in Phytophthora phaseoli during the infection of lima bean

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Phytopathology 101:S273

Lima bean is an important legume crop to the state of Delaware. This crop is susceptible to an oomycete pathogen, Phytophthora phaseoli, which caused significant crop loss in the year 2000. In this study we have used Illumina RNA-seq to identify genes in P. phaseoli orthologs to several effector genes in P. infestans, a close relative of P. phaseoli. To study the function of these effector proteins, we selected ten candidates with similarity to RxLRs, NPP1 and crinklers, all of which are different classes of effectors. Full-length sequences of most of the candidates showed more than 90 percent identity to amino acid sequences in P. infestans. The above effectors genes were validated by performing in planta RT-PCR. Phylogenetic analyses of candidate effector genes from other oomycete pathogens confirm a close relationship of P. phaseoli and P. infestans for all the corresponding effector genes. Selected effectors were cloned into Agrobacterium and injected into legume plants. Three elicitins, Pp_INF1, Pp INF4 and Pp 06908) showed a hypersensitive response. Currently, we are performing functional characterization of RxLR effectors and other effector genes, which will help us to gain a better understanding of this pathosystem and will serve as a basis for future research.
S274  PHYTOPATHOLOGY

Seasonal distribution of SI fungicide resistance in apple scab in Virginia S. C. MARINE (2), D. G. Schmale (1), K. S. Yoder (2) (1) PPWS Dept., Virginia Tech, Blacksburg, VA; (2) Virginia Tech AHS AREC, Winchester, VA Phytopathology 101:S274

Venturia inaequalis is the casual organism of apple scab, an economically devastating disease of apples that occurs where apples are grown. Management has predominantly relied on chemical applications, with sterol inhibitors like fungicides being one of the dominant systemic fungicides used in commercial apple production. Unfortunately, Virginia populations of V. inaequalis are developing resistance to myclobutanil and other SIs. We evaluated fungicide resistance in 266 single-spored V. inaequalis isolates collected in Winchester, VA between 2006 and 2010. Within a given season, the mean colony growth of V. inaequalis isolates was significantly different (P < 0.001) between treatments (50 ppm myclobutanil) and assay times (7, 14, 21 or 28 days). Whether the V. inaequalis isolate came from a treated or non-treated tree was significant in 2006 (P < 0.001) and 2008 (P = 0.002), but not in other years. Sampling interval was significant (P < 0.001) in 2007 and 2008. When analyzed concurrently, all factors were significant (P < 0.001) including collection year. Percent growth suppression (PGS) – the difference in colony growth on 0 and 1 ppm myclobutanil at 28 days – was used to assess fungicide resistance. Generally, a range of resistance was seen at each sampling interval, and the average PGS was similar for treated and non-treated trees of the same cultivar. In the May and June sampling intervals, the average PGS hovered around 60% regardless of cultivar or tree treatment. In contrast, the average PGS in the July sampling interval was around 30% (i.e. more resistant to myclobutanil). The average PGS in V. inaequalis in 2008, the year Mary did the treatments, was more similar to that seen early in the summer. High levels of fungicide resistance in populations of V. inaequalis suggest that replacement programs should be considered. Future research may rely on DNA-based methodologies to determine fungicide resistance and employ appropriate disease management strategies.

The molecular interaction of Theobroma cacao and Moniliophthora perniciosa, causal agent of witches’ broom, during infection of young pods R. L. MELNICK (2), J. Marelli (1), B. A. Bailey (2) (1) Mars Center for Cocoa Science, Fazenda Almairante, CP 55 Itajai Bahia 4630-000 BRAZIL; (2) Sustainable Perennial Crops Lab, PSI, USDA/ARS, Beltsville, MD, U.S.A. Phytopathology 101:S274

Infection of Theobroma cacao (cacao) flower cushions with the basidiomycete Moniliophthora perniciosa (Mp) can result in the formation of parthenocarpic fruits resembling cherimoya. Young green cherimoya-like fruits and healthy young cacao pods were obtained from 7 clones of different genetic backgrounds in Bahia, Brazil. QPCR analysis was conducted to determine how the infection process impacts the expression of both cacao and Mp ESTs. The effect of genotype was significant in the expression level of nearly all the August sampling interval (around 50%) was more similar to that seen early in the summer. High levels of fungicide resistance in populations of V. inaequalis suggest that replacement programs should be considered. Future research may rely on DNA-based methodologies to determine fungicide resistance and employ appropriate disease management strategies.

Inoculum density effects on infection of selected Eastern U.S. forest species by Phytophthora ramorum P. W. TOOLEY (1), M. Browning (1) (1) USDA-ARS, FDWSRU, Ft. Derrick, MD, U.S.A. Phytopathology 101:S274

Inoculum threshold information can be used to better understand the epidemiology of P. ramorum should it become established in the Eastern US. Detached leaves from Quercus prinus, Q. rubra, Acer rubrum, Kalmina latifolia ‘Hoffman’s K’, and Rhododendron ‘Cunningham’s White’ were exposed to sporangia concentrations ranging from 0 to 3000 sporangia/ml. Three leaves per species per experiment were dip-inoculated and positioned on top layers of moist paper towels in sealed plastic containers at 20°C for five days. Experiments were also conducted using stems of 2–3 year old seedlings of Q. prinus, Q. rubra and A. rubrum. Treatments ranging from 0 to 3000 sporangia/ml were applied to wounded and unwounded stem tissue. Detached leaf inoculations resulted in disease in all five species at all concentrations tested. Wounding was required for infection of stems to occur. With both stems and detached leaves, disease was observed at the lowest sporangia concentrations utilized; 100 sporangia/ml for stems and 10 sporangia/ml for detached leaves. The results indicate that P. ramorum is
capable of infecting some major Eastern U.S. forest species at low inoculum levels. This information can be combined with knowledge of understory host distribution and sporulation capacity to predict epidemic potential in new areas, and enhance development of risk assessment models.

Abscisic acid influences progression of a tomato disease caused by potato purple top phytoplasma

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Phytopathology 101:S275

Phytoplasmas are small, cell wall-less bacteria that are associated with numerous plant diseases. Plants infected by phytoplasmas often exhibit an array of symptoms consistent with hormone disorders. Significant changes in the endogenous levels of plant hormones upon phytoplasma infection suggest a role of plant hormones in the interactions of phytoplasmas with their plant host. Abscisic acid (ABA), one of the major plant hormones, is involved in various plant physiological processes. Conflicting evidence has been presented that ABA either promotes or represses plant resistance to pathogen infections. Results from our study using Columbia Basin potato purple top (PPT) phytoplasma and Rutgers tomato, as a model pathogen-host pair, revealed that i) endogenous levels of ABA surged at the early stage of phytoplasma infection and ii) exogenous administration of ABA delayed symptom development in PPT phytoplasma-infected tomato. Compared with control (ABA-untreated, PPT-infected) plants, appearance of the characteristic “big bud” symptom was significantly delayed and the number of “big buds” was significantly less in ABA-treated, PPT-infected plants. In addition, “little leaf” symptoms became unnoticeable in most of the ABA-treated, PPT-infected plants. Results of quantitative real time PCR analysis showed that, in scions, phytoplasma DNA titers remained higher in ABA-treated plants as compared to ABA-untreated plants 10 days post graft inoculation. One explanation of such phenomenon could be that ABA impeded phytoplasma movement from infected parts (scions) to healthy parts (root stocks). Similar results were also obtained from parallel experiments using an ABA bio-synthesis deficient tomato mutant as root stock. Our findings provide new insight into the role of ABA in phytoplasma-host interaction.

Gibberellin acid (GA3) treatment attenuates tomato floral deformation caused by potato purple top phytoplasma infection

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Phytopathology 101:S275

One of the most striking symptoms exhibited by potato purple top (PPT) phytoplasma-infected tomato plants is the formation of an aberrant floral structure known as “big bud”. Due to early abortion of pistils, stamens, and petals, the “big bud” structure is mainly composed of enlarged and fused sepals. It is apparent that PPT phytoplasma infection disrupts sequential expression of genes crucial for normal flower development. Since gibberellins are essential plant hormones that regulate flower morphogenesis, we were interested to learn whether exogenous application of gibberellin acid (GA3) would reprogram floral gene expressions and alleviate the “big bud” symptom. PPT-infected tomato plants were treated with 100 µM GA3 via leaf spraying two days after graft-inoculation. Developing bud samples were collected at four time points corresponding to four landmark stages in normal tomato flower development (stage 6, 9, 12 and 15) for morphological profiling and floral homeotic gene expression pattern comparisons. The morphological data from bud morphometric measurement and histological assay showed that GA3 treatment restored pistil, petal, and stamen development in PPT-infected tomato plants to levels comparable to those of healthy control plants. However, GA3 treatment did not significantly reduce sepal hypertrophy. Quantitative RT-PCR analysis of homeotic gene transcripts revealed that the expressions of class B and C genes LeAP3, FA and TAG1 were up-regulated at stages 6 and 9 in GA3-treated, PPT-infected plants (compared with PPT-infected plants without GA3 treatment), whereas the expression of class A gene was not substantially changed following GA3 treatment. In summary, treatment of PPT phytoplasma-infected tomato plant with 100 µM GA3 can partially restore floral organ development through transcriptional reprogramming of key floral homeotic genes.
2011 Caribbean Division Meeting Abstracts

Sorghum as a barrier and intercrop option against non-persistently transmitted viruses
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Phytopathology 101:S276

Watermelon, an important cucurbit crop in Puerto Rico, is susceptible to viral infections. Aphids and whiteflies vector different diseases in watermelon. To study the influence of Sorghum bicolor (L.) Moench as a border and intercrop in watermelon plots two experiments were planted in November 2009 and May 2010. Plots were sown in a randomized complete block design replicated four times. Populations of whitefly (Bemisia tabaci) were greater in 2009 when compared with 2010, and the treatment with sorghum intercrop resulted in the lowest number of adults of whitefly. During the third week of the crop cycle in 2010 significant differences in the whitefly populations were detected with a greater whitefly population in the control. The aphid (Aphis gossypii) population was not influenced by sorghum as a border or an intercrop. Presence of watermelon vine decline associated with a Potyvirus, Papaya ringspot virus and Zucchini yellow mosaic virus were assessed using DAS-ELISA tests during five weeks of the growth cycle. Results indicate these viruses were less frequent in 2010 than 2009. In the 2009 and 2010 experiments the border treatment yielded 26,580 kg/ha and 27,188 kg/ha, respectively. Sorghum intercrop and the control had an average of 56,943 kg/ha and 57,766 kg/ha in 2009, respectively, while in 2010 yields were 63,033 kg/ha with intercrop sorghum and 119,281 kg/ha in the control, respectively. These experiments demonstrated that used of sorghum did not contribute to the management of these viral diseases in watermelon. However use of sorghum as a barrier affected insect populations.

Characterization of endophytic bacteria isolated from healthy and diseased coffee trees showing witches broom and other symptoms under field conditions in Puerto Rico
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Phytopathology 101:S276

Coffee (Coffea arabica L.) an economic and socially important crop, generates revenues of $35 million for Puerto Rico in 2009. Main production problems are of biotic origin. Among bacterial diseases affecting this crop, coffee leaf scorch (CLS), caused by the bacterium Xylella fastidiosa (VF) Wells et al., is significantly important in many countries due to economic losses. In this study, we tried to characterize the bacterial species related to the vascular system of symptomatic and symptomless plants from coffee farms of the municipalities: Las Marias (18°13'14'', W66°01'38''), Adjuntas (18°09'30''W66°45'27''), Yauco (18°09'57'', W66°49'36'') and Jayuya (18°09'35'', W66°38'45''). In February, August, November and December of 2010, samples were collected from coffee trees showing marginal and apical leaf scorch, yellowing of new leaves, reduction of internode length, and abnormal production of new flushes (witches broom). Isolations on PW (periwinkle) medium were made from branches and leaf veins, to identify endophytic bacteria associated with the symptoms. Strains were differentiated by colony morphology, Gram stain, biochemical tests (oxidase, catalase), growth in TSA (tryptic soy agar), incubation period and in vitro pathogenicity tests. DAS-ELISA for Xf (AGDIA, Elkhart, IN) was performed for all Gram negative bacteria. Cluster analysis using the nearest neighbor method indicated that twice as many Gram negative bacteria of fastidious growth (PW+, TSA-) were found in diseased versus healthy trees.

Identification of powdery mildew in ornamentals and herbs in Puerto Rico
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Phytopathology 101:S276

Powdery mildew (Erysiphales) can cause economic losses on a wide range of ornamental plants. A survey was conducted in 2010/2011 in Puerto Rico. Symptomatic plants were collected and symptoms included spots of white to grayish mycelia on the leaves. The identification was carried out using light and scanning electron microscopy (SEM) and morphological characteristics such as conidal germination, germ tube morphology, fibrosin bodies, appressorial shape and the patterns on the outside of the conidia wall. To confirm fungal identity the internal transcribed spacer (ITS) region of rDNA was amplified with primers ITS1/ITS4 and sequenced. In Poinsettia (Euphorbia pulcherrima) Erysiphe australiana was identified based on the lobulate appresorial type, the terminal germination tube and using SEM where the end wall patterns are fibrillar and the turgid conidia surface is fluted. Neoxeysiphe sp. was found in Crape myrtle (Lagerstroemia indica) using SEM where a lineal pattern of the conidia surface and smooth of the lateral sides were observed. Results indicated that E. pisi infected Eryngium foetidium and Euphorbia heterophylla and sequenced-based identification showed a 93% and 97% identity respectively with reference ITS sequences of GenBank. In Oxalis barrelieri a 97% of identity was found with E. pisi. In Lablab purpureus, E. polygoni was identified and sequencing analyses indicated a 93% of identity. In Gerbera jamesonii, Podosphaera fusca with a 99% of identity after sequence analyses. The weeds E. heterophylla and O. barrelieri were not the source of inoculum of the powdery mildew found in Poinsettia and Crape myrtle respectively.

Weeds and insects with potential as hosts and vectors of disease agents prevalent on shaded coffee plantations in two localities of Puerto Rico
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Coffee (Coffea arabica L.) is the agricultural crop of mayor social and ecological importance of the mountainous area of Puerto Rico. Coffee production is concentrated in the central west of the island of P.R., which is composed of 21 municipalities. Weeds and their interaction with insects are potential problems in coffee plantations. Weeds can limit production and crop yield if not controlled. Insects represent another problem by direct damage or as vectors of diseases. In this study, a survey of weeds and insects found in coffee plantations under shade and sun was conducted to quantify and identify...
the species. Surveys were carried out during October and November 2010 in Adjuntas and Yauco, P.R. In Adjuntas, there were a total of 3,126 weeds counted under shade within the trees of Carbonero (Pithecellobium carbonorum), and Guaba (Inga vera) and under sun (control). In Yauco a total of 1,893 weeds under shade with Guaba (Inga vera) and under sun. The most abundant weeds of 5,019 counted were: Brachariaria purpurascenes, Fimbristylis miliacea, Commelina diffusa, Diefenbachia seguine and Spathodea campanulata. Over two hundred insects were found associated with the weeds such as Commelina diffusa, Impatiens walleriana, Brachariaria purpurascenes, Paspalum simum, Diefenbachia seguine, Eleusine indica L. and Echinocloa colona. Among the insects of major importance in coffee are the Hemiptera, family Cicadellidae. The most abundant species in the weeds were: Eutettix similis, Euhemiscorbia coffeacola, both are xylem feeders and potential vectors of Xylella fastidiosa and Bothriocera undata and Petrusa marginata both phloem feeders.

Disease incidence in Phaseolus vulgaris in the regions of Chiangia, Cuanza Sul and Malange, Angola

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In Angola, 70% of the common beans (Phaseolus vulgaris L.) are grown in the Northern and Central Provinces of Bié, Huambo, Huila, Kwanza Sul, Malange, and Uige. Approximately 240,000 ha of common beans are cultivated by subsistence farmers with average yields of 220 kg/ha (NEPAD-CAADD, 2005). A survey of diseases in nurseries, planted as part of a Pulse lidemuthianum Phaeoisariopsis griseola Huambo. Symptom recognition, humid chamber, serology and tissue the Instituto de Investigacão Agronómica (IIA), Plant Pathology laboratory in respectively, during 2009 and 2010. Disease identification was conducted at and Malange on the temperate Central Plateau and Northern Provinces, CRSP (USAID) project, was conducted during two visits to Huambo, Cela and Uige. Approximately 240,000 ha of common beans are cultivated by subsistence farmers with average yields of 220 kg/ha (NEPAD-CAADD, 2005). A survey of diseases in nurseries, planted as part of a Pulse CRSP (USAID) project, was conducted during two visits to Huambo, Cela and Malange on the temperate Central Plateau and Northern Provinces, respectively, during 2009 and 2010. Disease identification was conducted at the Instituto de Investigación Agronómica (IIA), Plant Pathology laboratory in Huambo. Symptom recognition, humid chamber, serology and tissue isolations on media were conducted. Angular leaf spot (ALS) caused by Phaeosariopsis griseola was prevalent on the local cultivar Olho de Perdiz and incidence was moderate in the nurseries. Anthracnose (Colletotrichum lindemuthianum), rust (Uromyces appendiculatus) and Ascochyta leaf spot (Phoma exigua) were identified with low incidence in the nurseries and in growers’ fields. Fusarium root rot, caused by Rhizoctonia solani, was also abundant in Malange in part due to excessive soil moisture. Pythium myriotylum, producing damping-off, was identified through DNA sequence analysis, and is the first report of this pathogen in common bean in Angola. There was also high incidence of common bacterial leaf blight (Xanthomonas axonopus pv. phaseoli) in a seed multiplication field. Bean Common Mosaic Necrotic Virus symptoms were observed as vein and stem necrosis in the Huambo nursery. Developing germplasm with resistance to these major diseases affecting beans in Angola is in process.

Fungi associated with roots and crowns of diseased wheat samples in Texas

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In Puerto Rico, bananas and plantains are important agricultural commodities; their combined production totaled 133,500 tons in 2008. Black and yellow Sigatoka leaf spot diseases, caused by Mycosphaerella fijiensis and M. musicola respectively, are responsible for significant losses of these crops in Puerto Rico, due to the high susceptibility of the most important cultivars. Consequently, new clones were introduced from Bioversity’s Musa International Transit Center in Leuven, Belgium. Clones were evaluated in the field over two crop cycles (2007–2010) for their responses to these diseases, as well as their agronomic and organoleptic traits. Clear differences in resistance were found among the clones. On a 0–6 scale (0 = no disease; 6 = >50% leaf area with lesions), mean disease severities at harvest ranged from 5.8 for the susceptible control ‘Grand Naine’ to 1.2 for FHA-18. Wide ranges were also observed in mean bunch weights (7.57 to 45.1 kg), numbers of hands (6.5 to 14.8), and numbers of fruit (57.0 to 263.7). Several clones, mainly from the Foundation Hondureña de Investigación Agrícola (FHLA), produced excellent resistance and agronomic traits (e.g. short pseudostems, large bunches and satisfactory organoleptic profiles) and could potentially replace susceptible cultivars in commercial production or play roles in a nascent organic market.

Identification of ammorphic powdery mildews on fruits and vegetables in Puerto Rico

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Powdery mildews (Ascomycetes: Erysiphales) are considered among the major plant diseases of economic importance in the world. In Puerto Rico the limited knowledge of the specific species of Erysiphales that affect our crops and their ecology worsen the plant-pathogen problems. In 2010 and 2011 we conducted surveys to examine powdery mildew diversity. Tissue samples from Abelmoschus esculentus, Carica papaya, Cucumis melo var. reticulatus, Cucurbita moschata, Cucurbita pepo, Lagenaria siceraria, Mangifera indica and Solanum lycopersicum, and the weeds: Euphorbia heterophylla, E. hirta and Sonchus oleraceus were collected in orchards and greenhouses throughout the island. Podosphaera sp., Oidium mangiferae, O. caricae, O. neolycopersici, Cystotheca lanestris and Erysiphe pisi were morphologically identified using light and scanning electron microscopy. Conidia germination, appressorium shape, fibrous bodies and patterns found on the outside wall of the conidia were the morphological criteria used for preliminary identification. Molecular identification was performed using PCR amplification of the ITS1-5.8S-ITS2 region of the rDNA. So far, Oidium neolycopersici was identified
affecting tomato using both techniques. This pathogen is closely related to North American *O. neolycopestici* and differs from *O. lycopersici* which is restricted to Australia. Critical identification of powdery mildews in Puerto Rico is of economic importance to prevent the introduction of new genera and species that could affect our crops and their ecology.

**Six decades with Julio Bird**

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In 1957 Dr. Julio Bird, returning home from Prof. Stackman’s plant pathology department in St. Paul, MN, visited me at Rockefeller University in New York City. I learned about his work with the raucesque (whitefly transmitted) viruses in Puerto Rico. His description of tropical plant virus diseases stimulated my own interest in this subject. In 1963 I got my first opportunity to come to Puerto Rico during a worldwide survey of tropical plant diseases, carried out by the Food and Agriculture Organization of the United Nations. This started a series of scientific visits to Julio at Rio Piedras and Mayaguez, where we shared our interest in insect vector interactions with tropical plants. Julio edited with me “Tropical Diseases of Legumes” (Academic Press, 1974) and we published several joint scientific papers. I accompanied Julio to make comprehensive observational and phenological studies, collect samples for electron microscopic examination and record by color photography the disease symptoms. Photography was our joint hobby. The originality of Julio’s ideas made his work with whiteflies outstanding, accounting for the extraordinarily wide scope of his achievements. But very few people knew that in addition to his research and teaching, Julio excelled as a sculptor and as an archaeologist. His keen observing powers permitted him to discover places where the prehistoric occupants of Puerto Rico gathered and where the remnants of their cultural traits and artifacts resulted in permanent changes of the flora. Traveling with Julio to different areas of the island, we found ornamented vessels, stone jewelry, and once an ancient burial place of the pre-Columbian inhabitants, only a few feet from the Munoz lighthouse. Where huge trees were uprooted to clear the land for new construction, Julio collected pieces of wood to transform them into exquisite sculptures. He donated several to me and I am treasuring them as a permanent reminder of my dear friend and admired scholar.

**Response of Phaseolus vulgaris lines to angular leaf spot**

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Common bean (*Phaseolus vulgaris L.*) production is affected by angular leaf spot (ALS) caused by *Phaeoasporiopsis griseola* (Sacc.) Fern. The objective of this study was to identify sources of resistance to ALS in cultivars from Angola. Inoculation of *P. griseola* on common bean genotypes was carried out with one isolate from Isabela, Puerto Rico in two separate experiments. *Phaseolus vulgaris* cv. ‘WM’-isolated and inoculated with an atorizamolin, and evaluated using the CIAT scale (1–9), where: 1 = resistant, 4 to 6 moderate resistant and 7 to 9 = susceptible. The isolate of *P. griseola* from Isabela infected bean lines of both Mesoamerican and Andean origin materials. In the first experiment, genotypes G05685 (Andean) and Flor de Mayo (Mesoamerican) were resistant to ALS. Montcalm, Amendoin and Canariensis had a low disease severity score that presented the highest disease severity. cultivars Catarina and Calembre were moderately resistant. In the second experiment, ALS disease severity scores did not increase compared to the first experiment. However, initial symptoms of ALS were observed within 10–12 days after inoculation, and cultivar Ermelinda showed a disease severity of 696 days after the inoculation, indicating moderate susceptibility to the *P. griseola* isolate from Isabela. Cultivar Armeño, Calindio, Chumbí, Quimbala, Oló and Perdiz, Pedro and Manteiga were also moderately resistant. Cultivar Canario had a low disease severity compared to the first experiment. The level of disease severity in the second experiment indicated that environmental conditions were not favorable for the disease.

**Phytopathogenic fungi as an alternative for the biological control of the invasive weed, Hyparrhenia rufa**

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*Hyparrhenia rufa* commonly known as Jaragua grass or thatching grass, is an aggressive weed that has invaded croplands in Puerto Rico. Several strategies have been proposed for its control such as the use of phytopathogenic fungi and small ruminants. During 2009, plots invaded by *H. rufa* were examined for disease symptoms. Plants were collected and fungi was isolated from leaf tissues. *Curvularia sp.* *Fusarium sp.*, *Sphaeroopsis sp.* and *Phoma sp.* were identified from leaf lesions. Pathogenicity tests conducted at greenhouse conditions demonstrated that all fungi identified were pathogenic to *H. rufa*, being *Phoma sp.* the most virulent. Future experiments will be expanded to test fungal pathogenicity to plant stems and panicles under greenhouse conditions and further evaluation of the potential biological control agents at field conditions.

**Detection of citrus greening with hi-fidelity PCR**

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In 2009, citrus greening caused by *Candidatus liberibacter asiaticus* (CLA) was identified in Puerto Rico. A survey on prevalence of CLA in citrus orchards on the island was conducted. After DNA extraction (DNeasy, Plant Mini Kit - QiAGEN) from symptomatic and asymptomatic leaves, Hi-Fidelity polymerase chain reaction (Hi-Fi-PCR) was compared with standard PCR for CLA detection in leaf midrib tissues. Amplification of DNA with PCR often resulted in false negatives (2.5 µl primers O11 and O12c, 12.5 µl of Go Green Tag, 2.5 µl molecular water). The Hi-Fi-PCR consisted of Buffer Mix: 1.75 µl; pNTP, 8 µl O11 and O12c, 12 µl molecular water; Enzyme Mix: 1 µl Taq, 0.2 µl Accuzyme; Template Mix: 5 µl PCR buffer (Accuzyme), 1 µl Enzime Mix 12.25 µl molecular water, for a final reaction of 50 µl of 29.75 µl DNA. The Hi-Fi-PCR was more sensitive for detection of CLA in contrast with the traditional PCR. From a total of 138 citrus samples, 11 samples amplified a 1160 bp band of the 16S rDNA with Hi-Fi PCR and no DNA amplified with traditional PCR. Accurate diagnosis is indispensable for the establishment of disease-free nurseries and orchards to implement control measures to prevent the spread of the disease.

**The search for resistance to a disease of common bean caused by a soilborne necrotrophic fungus**

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Bean cultivars with intermediate resistance and/or avoidance to white mold (WM) would reduce disease losses and require no input costs for growers. Thus, one goal was to identify sources of resistance in adapted and nonadapted common bean lines utilizing standardized greenhouse screening methods and field nurseries across major bean production regions. Multi-site testing facilitates identifying highly variable disease reactions encountered with this pathogen. A second project goal was to assess variation in common bean isolates of the necrotrophic pathogen *Sclerotinia sclerotiorum*. We devised a unique study on pathogen variation across bean-production areas that tests the hypothesis that pathogen variation within and between test sites influences identification of WM resistance. Mycelial compatibility groupings (MCGs), aggressiveness, and microsatellites are used to identify genotype and phenotype differences in the isolates that can influence stability of identified WM resistance over time and location. Collecting isolates from specific bean host lines replicated at each resistance screening site permitted us to assess within and between location variations. High variation in aggressiveness and genotypic variability (mean Mycelial compatibility groupings) of pathogen isolates within and between field screening nursery locations and greenhouse test isolates has been found. Another hypothesis we are testing is that isolates collected from screening nursery sites and greenhouse tests show similar phenotype and genotype variability as isolates collected from growers’ fields in the same region. When isolates from screening nurseries in each of 3 states were compared with grower field isolates in the same state, there were significant differences in aggressiveness. Our database of characterized isolates now facilitates new isolate characterization. The expected outcome is a set of characterized isolates for breeders and pathologists searching for unique and common clones with more or less aggressiveness to use in screening for resistance.

**Evaluation of biocontrol, coffee compost and Arachis glabrata on Phytophthora root rot in avocado**

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The effects of *Arachis glabrata*, coffee compost amendment, and biocontrol agents (*Bacillus subtilis*, and *Trichoderma harzianum*) on Phytophthora root-rot incidence in avocado were evaluated. The treatments were compared with an uninoculated control and a *Fosetyl-Al* treatment arranged in a randomized complete design with four replications using commercial organic growth
Vitro experiments demonstrated that artificial microorganisms’ populations significantly when applied, even when in vitro experiments demonstrated that T. harzianum was efficient to control P. cinnamomii mycelial growth. Soil respiration which was used as an indicator of suppressive conditions in growth media and pasteurized soil did not differ among treatments. The beneficial effects of coffee compost suppression of Phytophthora under field conditions should be explored further.

Survival of Bacillus thuringiensis and Bacillus pumilus, antagonistic bacteria to the coffee berry borer, in coffee trees under field conditions in Puerto Rico

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Coffee (Coffea arabica L.) is one of the most important crops in Puerto Rico (PR). Currently, the main problem in coffee is the coffee borer caused by the insect Hypothenemus hampei. The pest feeds and reproduces inside of the coffee berry, reducing its weight and quality, causing losses up to 50% of the harvest. The Bacillus group is one of the potential natural enemies that has been evaluated for the control of the coffee berry borer because it contains species with insecticidal properties. Bioassays were performed using the female insect with Bacillus thuringiensis (Bt) and Bacillus pumilus (Bp) treatments to determine their potential to control the mortality and movement. One strain of each species was selected based on insect mortality at 96h after inoculation. In year 2010, the selected bacterial strains were inoculated on coffee trees at flowering stage in Adjuntas, PR to determine their survival under field conditions. Four evaluations for colonizing bacteria were performed using stem and fruits samples from inoculated coffee trees. A RCB design and LDS were used to contrast the differences between total bacteria and Bacillus. Four bacterial collections were established containing strains with different pigmentation, gram positive bacteria and spore-forming Bacillus. A significant reduction of the total bacterial population from fruits was detected when treated with B. thuringiensis of 24 hours growth. Survival of both species was positive six months after inoculation although total bacterial populations including Bacillus showed a reduction six months after inoculation apparently caused by the high precipitation observed during September.

Endophytic bacteria from the vascular tissue of coffee (Coffea arabica L.) and citrus (Citrus sinensis L.) leaves found during the attempt to isolate the pathogen, Xylella fastidiosa in Puerto Rico

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Xylella fastidiosa (Xf) is the causal agent of coffee leaf scorch coffee and citrus variegated chlorosis. Xf is xylem limited and difficult to grow in artificial media. Periwinkle agar is semiselective for Xf, as other endophytes can grow on it. Bacteria with potential to interact with Xf can impact expression of disease and may represent biological control agents. Interactions of Xf with other endophytes have been reported including Methylobacterium extorquens (synergistic) and Methylobacterium mesophilicum and Curtobacterium flaccumfaciens (both antagonistic). Xylella fastidiosa and a list of the cultivable endophytes identified while attempting to isolate Xf in the vascular tissue of coffee and citrus are reported for the first time. Five hundred and sixty five isolates were obtained during over three years. Xf was detected using DAS-ELISA with a 75% in coffee and 6% in citrus. Fatty acid analysis, BIOLOG® and PCR were used for identification. Two species of Methylobacterium were found: M. mesophilicum with high frequency in both crops and M. extorquens found only in coffee. Curtobacterium flaccumfaciens, antagonistic to Xf, was found in low frequency and only in coffee. Frequent bacteria in coffee were: Bacillus cereus, B. coagulans, B. pumilus, Citrobacter farmeri, Curtobacterium pastinum, Erwinia stewartii ss. stewartii, Kurakia kristinae, E. varians, Methylobacterium mesophilicum, Microbacterium cholanatarum, Micromonas paracarbonacea, Pantoea dispersa, P. agglomerans (Erwinia herbicola), Pseudomonas amylofera, Psycrobacter immobilis, Staphylococcus aureus S. epidermidis, Staphylococcus simulans, Stenotrophomonas maltophilia, and Xanthomonas azonoptis pv. vasculorum. Frequent bacteria in citrus were: B. cereus, B. coagulans, M. mesophilicum, P. putida, Staphylococcus warneri, Stenotrophomonas maltophilia, and X. azonoptis pv. vasculorum.