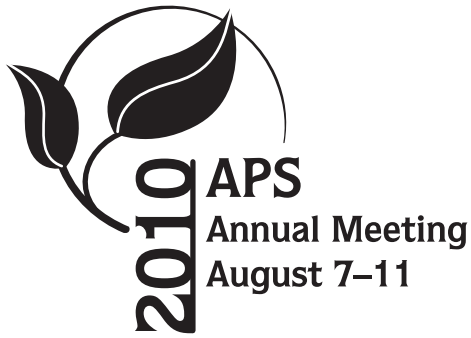


2010 APS Annual Meeting
Abstracts of Presentations

Abstracts submitted for presentation at the APS 2010 Annual Meeting August 7–11, 2010. The abstracts are arranged alphabetically by the first author's name.

Interception, identification and molecular characterization of three *Potato virus S* isolates infecting potato germplasm introduced from South America

J. A. ABAD (1), Y. Lin (2), C. J. Maroon-Lango (1), C. Loschinkohl (1), M. R. Smither (1), H. R. Pappu (2)
(1) USDA APHIS PPQ PHP Plant Germplasm Quarantine Program, Beltsville, MD, U.S.A.; (2) Plant Pathology Dept., Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S1

In the last three years, the USDA-APHIS-PPQ Plant Germplasm Quarantine Program intercepted several potentially highly infectious unknown and unusual virus isolates in potato germplam imported from South America. Recently, three PVS isolates, Q1, Q3 and Q5, were intercepted and characterized. The infected potato accessions were symptomless, and PVS was detected by bioassay, ELISA and molecular procedures. *Chenopodium quinoa* and *Nicotiana debneyii* showed symptoms following mechanical inoculations with Q1. However, Q3 and Q5 failed to produce symptoms in these plants. ELISA using PVS-specific antiserum was strongly positive for Q1 and inconclusive for Q3 and Q5. RT-PCR using *Carlavirus* generic primers was positive for all three PVS isolates. PVS-O-specific primer pair were positive for Q1 but negative for Q3 and Q5. Primers for PVS-A were negative for all isolates. The coat protein gene of each isolate was amplified, cloned, sequenced, and compared with those of known PVS isolates. Phylogenetic tree constructed using the CP amino acid sequences indicated that Q3 and Q5 clustered with the PVS-Andean group. The Q1 isolate was more closely related to the isolate Ha6-2 from Syria as well as U.S. isolates and clustered with other known PVS-Ordinary strains. Sequence alignments also suggested that isolates Q3 and Q5 have insertions in three regions of the CP gene when compared with the Vltava isolate of the Andean group of PVS.

Aflatoxin persistence in corn residues under no-till and conventional tillage management

H. K. ABBAS (1), R. M. Zablotowicz (2), C. Accinelli (3), C. A. Abel (4), N. A. Phillips (2), B. J. Johnson (2)
(1) USDA ARS CG&PRU, Stoneville, MS, U.S.A.; (2) Stoneville, MS, U.S.A.; (3) Bologna, ITALY; (4) Aimes, IA, U.S.A.
Phytopathology 100:S1

Little is known about the occurrence of aflatoxin in corn plant debris during the intercropping period. Corn was planted in two randomized complete block design experiments under no till (NT) and conventional till (CT) practices using Bt and Non-Bt corn in Elizabeth, MS in 2007 and 2008. Aflatoxin levels were determined at harvest and over-wintering corn within corn stover, cobs, and cobs with kernels. These plant components were collected from the soil surface of no-till (NT) plots or from the upper 5 cm of soil in conventional-till (CT) plots. At time of harvest, the aflatoxin in corn kernels from Bt and non-Bt hybrids were similar with greater aflatoxin found in grain from CT compared to NT plots. Likewise, a similar level of aflatoxin was found in various plant components of Bt and non-Bt residues. Regardless of genotype or tillage, the highest levels were found in cobs containing grain and the lowest content in stover. Aflatoxin concentrations in these tissues dissipated more rapidly in CT compared to NT plots. The levels found in these studies were much less than previously reported (Abbas et al., 2008), however, relatively high aflatoxin levels (100 to > 3000 ppb) persist in grain remaining on cobs under NT conditions one to seven months post-harvest.

Improving scab suppression and tuber yield of potatoes with multiple repeated applications of low rates of fish emulsion to a commercial field

P. A. ABBASI (1)
(1) Agriculture & Agri-Food Canada, London, ON, CANADA
Phytopathology 100:S1

Fish emulsion (FE), a liquid co-product of the processing of fish into fish meal, is an excellent organic product to enrich soil microbes and generate disease suppressive conditions against soil-borne diseases such as seedling damping-off, potato scab, and Verticillium wilt. Our studies suggest that biological control and organic acids play a role in FE-mediated disease suppression depending on substrates and soils. However, the broadcast rates (20,000 L/ha) of FE that provided effective control of potato scab may be too costly for commercial use. This long-term study was initiated to improve disease suppression and tuber yield of potatoes with multiple repeated applications of much lower broadcast rates (100 and 200 L/ha) of FE after harvesting and before planting potatoes starting in fall of 2007 for 3 years. Both rates (100 and 200 L/ha) of FE reduced scab severity by 34 and 42% in 2008 and 18 and 57% in 2009, reduced the percentage of tubers with deep-pitted scab by 23 and 30% in 2008 and 18 and 51% in 2009, and increased tuber yield by 16 and 19% in 2008 and 14 and 20% in 2009, respectively. FE soil amendment enhanced the numbers of soil bacteria including those of Pseudomonads and Bacilli. Lower rates applied more frequently may provide longer lasting disease suppression and may be economically feasible. FE is an excellent model system for development of an organic amendment to enhance biological control potential of natural soils.

Management of the root-knot nematode, *Meloidogyne incognita* on tomato in Egypt

M. M. ABD-ELGAWAD (1), S. S. Kabeil (2)
(1) National Research Center, Dokki, EGYPT; (2) Mubarak City for Scientific Research and Technology Applications, Alexandria, EGYPT
Phytopathology 100:S1

The efficacy of carbofuran at 1 mg a.i./kg soil, *Serratia marcescens* (1×10^9 bacterium cells/ml water) at 2 ml of the suspension/kg soil, and three different *Trichoderma harzianum* isolates each separately added at 50 ml./kg soil against the root-knot nematode *Meloidogyne incognita* on two tomato cultivars Super Strain B and Alisa was assessed in the glasshouse. Fresh and dry weight of shoots were higher ($P \leq 0.05$) in nematode-free plants of the two cultivars than both *M. incognita*-infested plants and the above-mentioned treatments. Carbofuran followed by *S. marcescens* and *T.*

harzianum generally decreased nematode development and reproduction parameters compared to the untreated control. Other effects of *M. incognita* infestation on protein content and enzyme activities are presented and discussed.

Impact of mycorrhizal infection on sensitivity of wheat to sorghum allelopathy

M. M. ABDELKARIM (1), R. J. Gualandi (1), K. D. Gwinn (1), B. H. Ownley (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 100:S2

Sorgoleone, an allelochemical exuded from the roots of sorghum, decreases growth of subsequent crops. The aim of this experiment was to determine impact of two species of mycorrhizae (*Glomus intraradices* or *Gigaspora margarita*) on allelopathic effects of sorghum (*Sorghum bicolor* Dekalb 'DK39Y') on wheat (*Triticum aestivum* Pioneer '26R22'). Sorghum plants were planted either in the presence or absence of mycorrhizae-infected sorghum roots. Vegetative growth of sorghum was removed after 12 weeks, and wheat (20 seeds/pot) was grown for one month in medium containing sorghum roots. The experiment had eight replicates with four treatments: no sorghum (control), non-mycorrhizal sorghum, *Glomus*-infected sorghum, and *Gigaspora*-infected sorghum. All growth parameters (shoot height, fresh shoot weight, fresh and dry root weights, and stem diameter) were greater for control than treatments that followed sorghum [$P < 0.0001$ for all parameters except stem diameter ($P = 0.009$)]. Plant height of mycorrhizal treatments was significantly higher than the non-mycorrhizal treatment, but there were no differences between mycorrhizal species ($P < 0.0001$). Root weight (fresh and dry) was greater in treatment with *Gigaspora* than in treatment with *Glomus*; neither was different from non-mycorrhizal treatment ($P < 0.0001$). In a natural infestation, the bird cherry oat aphid, *Rhopalosiphum padi*, was preferentially attracted to the non-mycorrhizal sorghum treatment (33.5 ± 8.1 aphids/plant); other treatments had fewer than one aphid/plant.

Two undescribed viral species isolated from native grapevines

N. Abou Ghanem-Sabanadzovic (1), S. SABANADZOVIC (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S2

More than sixty viruses have been reported from *Vitis vinifera* and related rootstock species/hybrids while very little is known about viruses infecting grape germplasm native to the American continent. A survey aimed at the identification of viruses present in native *Vitis* germplasm in the Southeastern U.S.A. was carried out in 2007 and 2008 exclusively targeting wild specimens. Shotgun sequencing of reverse-transcribed dsRNAs extracted from a specimen of *Vitis aestivalis* collected from Great Smoky Mountains National Park revealed co-infection by two unrelated viruses. Complete sequences were generated for both viruses: i) a multipartite dsRNA virus related to *Rice ragged stunt virus* (gen. *Oryzavirus*; fam. *Reoviridae*) and ii) a luteovirid with similarities with *Pea enation mosaic virus 1* (PEMV-1; gen. *Enamovirus*; fam. *Luteoviridae*). While the oryza-like virus was detected in only one additional specimen, enamo-like virus seems to be rather widespread in the native grapes. No members of these taxa were previously reported from grapevines. Susceptibility of cultivated species/hybrids to these viruses is yet to be examined.

Detection of toxin and non-toxin forms of *Aspergillus flavus* and *A. parasiticus* by RT-PCR in Georgia peanuts

P. ACHAR (1)
(1) Kennesaw State University, Kennesaw, GA, U.S.A.
Phytopathology 100:S2

Aspergillus flavus and *Aspergillus* parasiticus are common contaminant in peanuts and are responsible for the production of carcinogenic aflatoxin. PCR are routinely used to detect *Aspergillus* spp. in peanuts. In the present study Real-Time PCR (RT-PCR), was used to detect aflatoxigenic from non-aflatoxigenic strains. Universal primers (ITS) 1 and (ITS) 4 were used to amplify a conserved region of the internal transcribed space for the non-toxin producing sequences. Specific primers, *Nor* 1 & 2 and *Ver* 1 & 2, were used to identify the toxin producing sequences. The Roche Light Cycler 480 and SYBR Green were used for RT-PCR. The melting points gave insight into the amplicon length and G-C content of both spp. In all samples tested, the ITS gene concentration was a factor of 181 and 1,024 times more than the Nor and Ver genes amplified, respectively. PCR products with ITS primers ranged from 550-600 bp while Nor at 350 bp and Ver at 475 bp using 2.0% agarose gel. Our results showed the ability of RT-PCR to detect the presence of toxin and non-toxin producing genes in *Aspergillus* spp. Relative quantification supported the prediction that a greater concentration of housekeeping genes would be present in all the samples compared to the genes for aflatoxin biosynthesis. Our results indicated that RT-PCR is faster and more accurate than traditional methods and hence recommended for early detection of aflatoxin strains in peanuts since aflatoxin is a potential carcinogen.

Tracing the emergence of resistance breaking variants of *Beet necrotic yellow vein virus* in nature

R. ACOSTA-LEAL (1), B. K. Brian (2), C. M. Rush (2)
(1) AgriLife Research (Texas A&M), Amarillo, TX, U.S.A.; (2) Texas AgriLife Research, Amarillo, TX, U.S.A.
Phytopathology 100:S2

The capability of BNYVV to overcome the sugar beet *Rz1*-mediated resistance is frequently associated with the presence of a specific amino acid in the hypervariable region of the viral p25 (RNA-3). The underlying C to U mutation has independently occurred in Minnesota and California where resistance breaking (RB) variants cause characteristic yellow spots of *Rz1*-plants in the field. Multiple yellow spots often develop in a single field. Given the nature of the RB mutation, the objective of this work was to determine if RB variants can emerge in parallel multiple times from sympatric avirulent haplotypes during the same crop season. A fragment of the viral RNA-3 from single-plant isolates was cloned and sequenced from several *Rz1*-plants inside and outside yellow spots to analyze the genetic composition of these populations. Co-infections of avirulent and RB haplotypes were commonly found in diseased plants and occasionally in asymptomatic plants. Plants close together were sometimes infected by the same predominant haplotype. Several avirulent progenitors have been identified and two evolutionary pathways to overcome *Rz1* apparently have taken place in the Imperial Valley.

Selection of isolates of *Penicillium expansum* with reduced sensitivity to fludioxonil and pyrimethanil from sensitive, single-spored isolates

J. Adaskaveg (1), H. FORSTER (2)
(1) University of California, Riverside, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.
Phytopathology 100:S2

Isolates of *Penicillium expansum* with reduced sensitivity against the two new postharvest fungicides fludioxonil and pyrimethanil could be readily obtained when large numbers of conidia (10^8/plate) of single-spored sensitive isolates were plated on selection plates. Agar plates contained a continuous concentration gradient for each fungicide. There was no correlation between the number of resistant isolates obtained and the degree of heterogeneity of the sensitive population (1, 4, or 60 isolates) that was used in the selection assays. Resistance frequencies ranged from 1×10^{-8} to 3.6×10^{-5} for fludioxonil and from 1.2×10^{-8} to 1.8×10^{-6} for pyrimethanil. For fludioxonil, isolates were either moderately resistant (EC_{50} 0.77 to 3.5 mg/L; sensitive isolates: <0.02 mg/L) or highly resistant (EC_{50} >40 mg/L), whereas for pyrimethanil a range of sensitivities (EC_{50} 1.8 to >75 mg/L; sensitive isolates: <0.70 mg/L) was observed. Isolates insensitive to both fungicides were recovered at very low frequency in some tests and always displayed a lower level of resistance. Most resistant isolates were stable in culture and were pathogenic in apple fruit inoculations. In these experiments, no isolates with reduced sensitivity to difenoconazole, another fungicide planned for registration, were obtained. Our data indicate that the risk of resistance development against new postharvest fungicides for pome fruit is high and that resistance management is crucial.

Competitiveness of *Penicillium expansum* isolates with reduced sensitivity to fludioxonil and pyrimethanil during infection of apple fruit

J. Adaskaveg (1), H. FORSTER (2)
(1) University of California, Riverside, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.
Phytopathology 100:S2

Isolates of *Penicillium expansum* resistant to the new postharvest fungicides fludioxonil and/or pyrimethanil and pathogenic to pome fruit were selected in the laboratory from natural populations of the pathogen. The competitiveness of these isolates was evaluated in co-inoculations of apple fruit with a sensitive wild-type isolate of *P. expansum*. Competiveness was based on the proportion of resistant to sensitive progeny that were grown from conidia collected from decaying fruit. Either of two isolates highly resistant to fludioxonil (EC_{50} >40 mg/L as compared to <0.02 mg/L for sensitive isolates) were not recovered after co-inoculation with the sensitive isolate, whereas when using an isolate highly resistant to pyrimethanil (EC_{50} >75 mg/L as compared to <0.70 mg/L for sensitive isolates) 27.1 to 33.3% of conidia from decaying fruit displayed the resistant phenotype. In co-inoculations with either of two isolates of P. expansum with an intermediate level of resistance to both fungicides (EC_{50} 0.12 or 2.42 mg/l for fludioxonil, 1.74 or 2.08 mg/L for pyrimethanil), 22.9 to 35.4% of the collected conidia displayed the double-resistant phenotype. These data indicate that differences in competitiveness exist among resistant isolates, that with repeated fungicide applications some isolates may become predominant, and that proper anti-resistance strategies have to be followed in the use of these new fungicides.

Evaluation of winter wheat accessions for resistance to *Xanthomonas translucens* pv. *undulosa*

T. B. ADHIKARI (1), J. Hansen (1), S. Gurung (1), J. M. Bonman (2)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, U.S.A.
Phytopathology 100:S3

Bacterial leaf streak (BLS) caused by *Xanthomonas translucens* pv. *undulosa* (Xtu), has re-emerged and become an important disease of wheat in the northern Great Plains of the United States. Breeding for disease resistance and planting resistant varieties offers the best approach to control BLS in the absence of effective bactericides. Most of the currently grown wheat varieties in the United States appear to be susceptible to BLS. Over 400 winter wheat accessions from a core subset of the USDA National Small Grain Collection, representing landraces, cultivars, and breeding lines of diverse origin, were assessed for resistance to BLS at the flag leaf stage during 2009 and 2010 in a greenhouse at NDSU. Results showed that the wheat accessions have a wide range of susceptibility to BLS but 41 accessions exhibited resistance to the disease. Resistance was significantly more frequent in accessions from North America, Australia-New Zealand, Western Asia, and South-central Asia compared to accessions from Northern and Eastern Europe. The United States had the most resistant accessions followed by China and Germany. There was no association between kernel color or accession improvement status (e.g., landrace, cultivar, breeding line) and BLS resistance. Overall, this study identified potential new sources of resistance to BLS, which could be utilized in winter wheat improvement programs.

Potential viral threats to *Miscanthus* x *giganteus* and switchgrass production for bioenergy in the United States

B. O. AGINDOTAN (1), M. O. Ahonsi (2), M. E. Gray (2), C. A. Bradley (2)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) Energy Biosciences Institute, University of Illinois, Urbana-Champaign, Urbana, IL, U.S.A.
Phytopathology 100:S3

Miscanthus x *giganteus* and *Panicum virgatum* (switchgrass) are two potential biomass crops being evaluated for cellulosic ethanol production. Because they could be cultivated in large scale for biomass purposes, it is important to identify viruses that are potential threats to these crops. Identification of viruses infecting these crops is important for quarantine purposes, virus resistance breeding, and production of virus-free planting materials. Using a combination of methods, viruses were identified that infected these crops in fields in Illinois, Iowa, Wisconsin, Kentucky, Tennessee and Georgia. The most common viruses infecting both *M.* x *giganteus* and switchgrass were *Sugarcane mosaic virus* (SCMV) and *Wheat soil-borne mosaic virus* (WSBMV). *Barley yellow dwarf-PAV virus* (BYDV-PAV) was detected only in switchgrass in Illinois. A new virus of switchgrass was identified, which was closely related to, but significantly different from *Maize rayado fino virus* (MRFV). Other virus-like symptoms were observed on collected samples of *M.* x *giganteus* and switchgrass, and identification of causal agents is in progress.

Isolation and characterization of *Pectobacterium carotovorum* mutants host-signal responsive genes

P. A. AGYEMANG (1)
(1) Tennessee State University, Nashville, TN, U.S.A.
Phytopathology 100:S3

Pectobacterium carotovorum (*Pc*) is a phytopathogenic bacterium that causes soft rot disease in economically important crops by producing extracellular enzymes triggered by its host signals and other environmental cues. Enzyme production and pathogenicity depends on a coordinated regulation of a number of genes in the pathogen. Approximately 20% of the putative proteins of *Pc* have unknown function. *Pc* strain AC5006N3 (lacZ$^-$, Nal^R) was mutagenized using mini-Tn5 Km containing a promoterless lacZ reporter. About 31,122 colonies were screened in the presence and absence of celery extract (CE) to obtain mutants in the host extract-regulated genes. After repeated screening and evaluation of promoter activity of the mutants based on betagalactosidase (β-gal) assays, 98 mutants were obtained of which 83 were induced (> 5-fold) and 15 were repressed (<0.5-fold) in the presence of CE. Semiquantitative assays on selected (26 induced and 3 repressed) mutants showed increased production of cellulase (Cel), pectate lyase (Pel) and protease (Prt) in the presence of CE. Quantitative assays revealed that, compared to the parent, 6 of these mutants overproduced (> 2-fold) Pel while one was deficient (< 0.5-fold). Three mutants overproduced Prt while 5 were deficient (< 0.5-fold). In a pathogenicity test using celery petioles and potato tubers, 6 mutants macerated at least 2-fold more tissues compared to the parent. Nine mutants had reduced ability to macerate the tissues. Identification of genes of unique mutants is imperative.

Grapevine necrotic union, a newly recognized disease in grapevines on 110 Richter rootstock in California

M. Al Rwahnih (1), A. Rowhani (1), R. J. Smith (2), J. K. Uyemoto (3), M. R. SUDARSHANA (3)
(1) University of California, Davis, CA, U.S.A.; (2) University of California Cooperative Extension, Santa Rosa, CA, U.S.A.; (3) USDA ARS, Davis, CA, U.S.A.
Phytopathology 100:S3

In early fall of Yr 2004, inspection of a 7-year old vineyard of Pinot noir (PN) clone 02A (Vitis vinifera L.) grafted on rootstock 110 Richter (110R; V. berlandieri x V. rupestris) in Sonoma County, CA, revealed ~ 2.1% of the grapevines with symptoms of solid red leaf blades, weak shoot growth and grape clusters with reduced set. Examination of trunk specimens indicated a necrotic line at the scion-rootstock junction and hence named "grapevine necrotic union" (GNU). Yearly surveys indicated that GNU incidence gradually increased to 22% in Yr 2009. This disease was also observed in PN clones 02A (PN02A), 04, 667, and 777, in Napa County, and Pinot gris 152 on 110R in Monterey County, and the incidence ranged from 2.0% to 45%. RT-PCR assays did not indicate any known grapevine viruses that could be considered associated with diseased vines. Repeated chip bud grafts of diseased vines onto test plants of Cabernet Sauvignon 08 on 110R rootstock failed to demonstrate a graft-transmissible agent. However, bench grafts of several PN clones and Chardonnay-04 (Ch-04) produced GNU on 110R but not on rootstock 3309 Couderc (V. riparia x V. rupestris). Ultradeep sequencing analysis of cDNA made from dsRNA obtained from bark scrapings indicated the association of Grapevine redglobe virus and Grapevine rupestris vein-feathering virus in Ch-04 and PN02A vines. These two viruses were also found associated with GNU affected vines in Napa County.

Characterization of pervasive latent viral infection of olive trees in the National Clonal Germplasm Repository

M. AL RWAHNIH (1), Y. Guo (1), S. Daubert (1), D. Golino (1), A. Rowhani (1)
(1) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 100:S3

The olive industry in California is booming. Oil olive acreage is growing at 4000 acres per year, and acreage of table olives is also expanding. Propagation material is being requested in ever-larger quantities from the U C Davis-USDA National Clonal Germplasm Repository, which is recognized as one of the richest collections of olive material in California. The repository maintains 107 different olive varieties, imported from nineteen different countries. However, the collection at the Germplasm Repository has never been systematically characterized as to its viral infection status. We have now completed the first comprehensive virus testing of the collection using molecular diagnostic tools, and have compiled a list of the viruses that were detected. A total of 54 trees from 36 different cultivars were sampled. Though these trees were asymptomatic, the samples from 97.9% of them showed dsRNA profiles indicating viral infection. 91.5% of those trees tested positive for *Olive leaf yellowing-associated virus* (OLYaV) by RT-PCR analysis, while 39.5% were positive for *Cucumber mosaic virus*. PCR amplicons of the OLYaV HSP70h gene were cloned and sequenced to analyze the molecular variability between isolates from trees originating from different geographical regions. The sequence analysis showed a maximum of 27% divergence between amplicons obtained from these selections.

Profiling host-specific and virus-derived small RNAs in a woody perennial plant species infected with an ampelovirus

O. J. ALABI (1), Y. Zheng (2), G. Jagadeeswaran (3), R. Sunkar (3), R. A. Naidu (4)
(1) Washington State University, Prosser, WA, U.S.A.; (2) Institute of Developmental Biology and Molecular Medicine and School of Life Sciences, Fudan University, Shanghai, China, 200433, Shanghai, PRC PEOPLES REP OF CHINA; (3) Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK, U.S.A.; (4) Department of Plant Pathology, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S3

Grapevine leafroll disease (GLRD) is an economically important viral disease of wine grapes. In this study, we used Solexa deep sequencing technology to compare small RNA (sRNA) profiles between GLRD-affected and unaffected leaves. Total RNA extracted from symptomatic leaves tested positive for *Grapevine leafroll-associated virus 3* (GLRaV-3, genus *Ampelovirus* and family *Closteroviridae*) and uninfected leaves were used for constructing small RNA libraries and sequenced. Approximately 2.3 and 1.5 million small RNA reads of 18 to 28 nt length were obtained from uninfected and infected libraries, respectively. Sequence analysis indicated that the host microRNAs (miRNAs) and other endogenous small interfering RNAs (siRNAs) were differentially regulated in infected samples. In addition, sRNAs derived from

GLRaV-3 were identified in the infected sample. Virus-derived sRNAs were mapped to the genome of GLRaV-3 according to their polarities and the results suggested the presence of potential hotspots that generate siRNAs from the virus genome. To our knowledge, this is the first study to profile miRNAs in closterovirus-infected perennial crop by deep sequencing approach.

Genetic variation of *Sclerotinia sclerotiorum* on four crops from the north central United States

L. ALDRICH-WOLFE (1), S. E. Travers (2), B. D. Nelson (3)
(1) Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.; (2) Biological Sciences, North Dakota State University, Fargo, ND, U.S.A.; (3) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S4

Sclerotinia sclerotiorum is a pathogen of many commonly-grown crops in the north central United States, yet little is known about genetic variability in the region and across crops. In 2008 149 isolates were collected from four crops (canola, dry bean, soybean, and sunflower) in twelve North Central states and WY, MT and CO. Isolates were evaluated for mycelial compatibility group (MCG) and microsatellite haplotype at twelve loci. Forty-six MCGs were identified. The most common was MCG 9 found in nine states and on all four crops. Six of the most common MCGs represented 58% of the isolates and were found across crops. There was a strong association between MCGs and microsatellites. For example, all isolates within MCG 9 shared a single microsatellite haplotype, while in MCG 8 there were three microsatellite haplotypes. To date, MCGs do not appear to be restricted to particular geographic regions or host crops. Gene diversity was as high among samples from eastern North Dakota and western Minnesota as among all samples, suggesting the pathogen may be as diverse genetically in local areas as across broad geographic regions. Microsatellite analysis found no evidence for differences in genotype or genetic diversity of the pathogen among the crops. Within eastern North Dakota and western Minnesota (where crops were more equally represented in the samples), gene flow appeared to be high between soybean and dry bean and lowest between sunflower and the two legumes.

Selection indexes comparison to improve maize resistance to *Aspergillus flavus*, *Fusarium moniliforme* and *Rhizoctonia solani* grain and plant infection

J. M. ALEZONES (1), R. D. Perdomo (1), B. Borges (1), A. D. Gonzalez (1), J. J. Salazar (1), F. R. Hernandez (1)
(1) Fundacion Danac, San Felipe, VENEZUELA
Phytopathology 100:S4

Preharvest contamination of maize by *Aspergillus flavus* (AF) and *Fusarium moniliforme* (FM) mycotoxins is considered a serious health problem. *Rhizoctonia solani* (RS) is also a harmful pathogen for this crop in several countries. The identification of cultivars with adequate performance in multiple traits is important in any breeding program, and for that, several selection indexes (SI) have been developed. In order to identify which index is more suitable for selection to these diseases, a two year trial was conducted involving the evaluation of 740 inbreds. To evaluate AF and FM resistance, kernel screen assay (KSA) technique was used, for RS a screening technique involving inoculation of plants with rice grains colonized by the fungus was performed. Genetic progress percent (GP), a "balance value" (BV), that is a relation between the trait with less advance and the one with the biggest progress, and the product of these two variables "PB", were calculated for five SI (Classic, Base, Free of weights, Multiplicative and Sum of Ranks) under a selection fraction of 25%. For both years the SI with the highest GP was the "Classic", but it posses a great lack of balance, which means that some diseases have a great advance when others keeps the same. On the other hand the "Sum of ranks" index was the one with higher BV. Finally, when PB is calculated, Sum of Ranks index posses the higher value, meaning that GP and BI for this index were simultaneously high, making it suitable for selection to these diseases.

Pustule density and latent period of *Puccinia hordei* on Iraqi barley genotypes

M. AL-HAMDANY (1), M. M. Salih (1)
(1) Ministry of Science and Technology, Baghdad, IRAQ
Phytopathology 100:S4

Disease severity of *Puccinia hordei* on barley genotypes and latent period were thoroughly investigated under field and growth room conditions respectively in Baghdad region. Barley genotypes included 9 cultivars, 13 induced mutants, 8 selected germplasims, and 300 M2 variants following gamma irradiation on Numar cultivar were used. Data of pustule density on these genotypes under artificially heavy epidemic form, indicated that disease responses of all tested genotypes could be classified to four groups: Highly susceptible such as cultivars Golden melon, Aimer, Beacher, Weah and Arivat, mutants D/21, D/30 and D/32; Susceptible such as cultivar Prior, mutants D/24, C/50, NA/20, and C/63, selected germplasms 480, 552, and 557; Moderate susceptible as in cultivar Gazera 2, the mutants D/34, TB/5 and VB/7, and selected germplasms 102, 576, 577 and 657 and mildew resistance source H-421; Moderate resistance included both cultivars Gazera 1 and Numar, the mutants OA/15, VB/6, and SA/12, all M3 Numar variants regardless the dose used of gamma rays. The latent period of *P. hordei* on detached leaves was 132 hrs. in the first two groups, 156 hrs. in the third and 168 hrs. on barley genotypes might have a partial resistance to *P. hordei* in Iraq. However, promising new source leaf rust resistance was identified.

Frequency of 3ADON and 15ADON isolates of *Fusarium graminearum* from field plots of wheat inoculated with mixed pathogen populations in North Dakota

S. ALI (1), K. Puri (1), M. McMullen (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S4

Isolates of *Fusarium graminearum* can be identified as one of the three chemotypes, 3-acetyl-Deoxynivalenol (3ADON), 15-acetyl-Deoxynivalenol (15ADON) and nivalenol (NIV) based on their trichothecene profile. Recently, 3ADON isolates have increased in the fungal population in the Northern Great Plains of the U.S. and Canada; the reason for the population change is still not known. We inoculated FHB susceptible (Briggs) and moderately resistant (Alsen) spring wheats with individual populations of 3ADON and 15ADON chemotypes (ten isolates each) and a mixed population (ten isolates of each chemotype), and added a flowering-time fungicide treatment, under field conditions. *F. graminearum* isolates (575) were randomly recovered from the inoculated plots and analyzed for chemotype using polymerase chain reaction (PCR). Results showed a significantly higher recovery frequency of 3ADON isolates than of 15ADON isolates in the mixed population inoculated on Briggs, with or without fungicide treatment, and on Alsen without fungicide treatment. No significance was observed in recovery frequency of the two chemotypes from mixed-inoculation on Alsen with fungicide treatment, but the number of isolates recovered from this treatment was low (34). The results suggest that the 3ADON isolates may be more competitive than the prevalent 15ADON isolates under North Dakota field conditions, regardless of cultivar resistance or use of fungicide.

Viruses infecting cucurbit crops in Oklahoma

A. ALI (1), A. Khattab (2)
(1) University of Tulsa, Tulsa, OK, U.S.A.; (2) Department of Biological Science, The University of Tulsa, Tulsa, OK, U.S.A.
Phytopathology 100:S4

During the growing season of 2008, surveys were conducted to detect and determine the incidence of viruses in the major cucurbit growing areas of Oklahoma. A total of 588 symptomatic leaf samples from 36 fields in 3 counties (Atoka, Blaine and Tulsa) were collected from five cucurbit crops (cantaloupe, cucumber, pumpkin, squash and watermelon). All samples were tested by dot-immuno-binding assay (DIBA) against the antisera of seven viruses, including *cucumber mosaic virus* (CMV), *cucumber green mottle mosaic virus* (CGMMV), *melon necrotic spot virus* (MNSV), *papaya ringspot virus-watermelon strain* (PRSV-W, *squash mosaic virus* (SqMV), *watermelon mosaic virus-2* (WMV-2) and *zucchini yellow mosaic virus* (ZYMV). The highest incidence was recorded for PRSV-W, followed by WMV-2, and ZYMV, which were contained in 67%, 17% and 15% respectively of the collected samples. MNSV, SqMV, and CMV were detected in 6.0%, 5.2% and 1.2% of the samples, respectively. None of the samples reacted positively against the antiserum of CGMMV. Mixed virus infections were common involving two or three viruses in various combinations. Triple and double infections were found in 6.8% and 5.6% of samples, respectively. Some symptomatic samples of watermelon, squash and pumpkin did not react with the antiserum of the above tested viruses, indicating that other unknown viruses may be infecting cucurbit crops.

Incidence and genetic variation of *Tobacco mosaic virus* through some tomato fields of Tehran

A. ALISHIRI (1), F. Rakhshandehroo (1), H. Zamanizadeh (1)
(1) Department of Plant Pathology, Islamic Azad University, Science and Research Branch, Tehran, IRAN
Phytopathology 100:S4

Tobacco mosaic virus (TMV) is a type member of the genus *Tobamovirus*. This virus is one of the most destructive viral diseases of plants. The goal of this study was to determine the incidence of *Tobacco mosaic virus* (TMV) on tomato plants. During the years 2009 to 2010, a survey was conducted through different tomato fields. A total of 256 leaf samples were randomly collected from symptomatic and asymptomatic tomato plants in four zones of the Tehran province of Iran and tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific polyclonal

antibody (Agdia, U.S.A.). Results showed that 10.6% of the tested leaf samples are infected with the TMV. Using PCR molecular method and specific primers designed for the coat protein of the viral genome sequence, presence of the TMV was confirmed for the ELISA positive-tested tomato plants. All infected tested samples amplified a 694-bp fragment in PCR reaction. The amplified fragments of five isolates have first been sequenced and then aligned with the corresponding data available for other TMV isolates in NCBI. Phylogenetic analysis revealed that one of all these Iranian isolates together with some NCBI isolates were categorized in one cluster while all other TMV isolates from NCBI were categorized in a separate cluster. Further studies on the distribution of this virus in different provinces is, now undertaken.

Effect of an at tassel fungicide application on yield and the foliar disease complex in field corn in Mississippi

T. W. ALLEN (1), A. Henn (2), D. M. Ingram (3)
(1) Mississippi State University, Stoneville, MS, U.S.A.; (2) Mississippi State University, Starkville, MS, U.S.A.; (3) Mississippi State University, Raymond, MS, U.S.A.
Phytopathology 100:S5

Over the past few seasons foliar fungicide applications have drastically increased across the United States in field corn in response to reports that an automatic fungicide application will result in increased yield in the absence of yield-limiting foliar diseases. Fungicides are suggested in the absence of yield-limiting diseases and applications are timed for tassel (VT). In response to this growing practice, research was conducted in Mississippi from 2007 to 2009 using three fungicides. Labeled rates of azoxystrobin + propiconazole (as Quilt), propiconazole (as Propimax), and pyraclostrobin (as Headline) were applied at VT at 27 locations, both in large (by air, n = 16) and small plot trials (by ground, n = 11), over the three year period and replicated at least 4 times at each location. In addition to collecting yield data at the end of the season, plots were rated post-application for disease incidence and severity as well as destructive plant sampling to determine potential impacts of the fungicides on overall plant health. Plants destructively sampled were rated for incidence of root and stalk diseases. In general, foliar disease incidence was less than 5% at all locations for the three year study. Collectively, fungicide application did not significantly increase yield compared to the untreated plots for any of the chemistries utilized.

Characterization of promoter elements from plant pararetroviruses associated with dahlia (*Dahlia variabilis*)

C. V. ALMEYDA (1), K. L. Druffel (1), H. R. Pappu (1)
(1) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S5

Three distinct caulimoviruses have been reported from dahlia: *Dahlia mosaic virus* (DMV), Dahlia common mosaic virus (DCMV) and an endogenous plant pararetrovirus (DMV-D10). Based on sequence comparisons and promoter prediction programs, the putative 35S promoter region from these three viruses was identified. The promoter regions were independently cloned into pCAMBIA1281Z. All constructs were introduced into *Agrobacterium tumefaciens* by electroporation, and agroinfiltrations were done into *N. benthamiana*. The activity and strength of the putative 35S promoter was determined by transient expression of the beta-glucoronidase gene (GUS). Results from qualitative GUS assays demonstrated that DMV, DCMV and DMV-D10 promoter activity is similar to that of *Cauliflower mosaic virus* 35S promoter.

Metagenomic analysis of mycoviruses in grapevines

M. ALRWAHNIH (1), S. Daubert (1), A. Rowhani (1)
(1) University of California, Davis, CA, U.S.A.
Phytopathology 100:S5

Fungal viruses may be at least as numerous as plant viruses, but mycoviral ecology and diversity is as yet largely unstudied. We have employed a metagenomic approach to characterize the mycovirus populations on grapevines. This approach was chosen to avoid the exclusion from our analysis of viruses from non-culturable, non-purifyable fungal host species. Total double stranded RNA was isolated from grapevine stem tissue and subjected to analysis by 454 high-throughput sequencing and BLAST screening. The analysis revealed a set of prevalent, diverse sequences related to mycoviruses. Twenty four putative mycoviral groups were identified in the samples, representing half of all known mycoviral families including the *Chrysoviridae*, *Hypoviridae*, *Narnaviridae*, *Partitiviridae*, and the *Totiviridae*. The presence of the viral genomes identified by the BLAST screening was confirmed by PCR tests of the starting stem tissue extract using primers designed from the viral sequences identified in the informatic analysis. Three of the putative mycovirus species were associated with *Botrytis cinerea*, a fungal pathogen of grapes. But most of the species were found to be undescribed mycoviruses and identified only through their distant sequence relationships with known mycoviruses from non-grapevine fungal hosts.

Effect of temperature and UV radiation on survival of *Coniothryrium minitans* and on efficacy in controlling lettuce drop caused by *Sclerotinia minor*

M. ALVARADO-HERNANDEZ (1), B. M. Pryor (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S5

Coniothyrium minitans has been previously reported to be more effective as a biocontrol agent against *Sclerotinia sclerotiorum* than *S. minor*. It was suspected that environmental factors played a major role in reducing *C. minitans* survival and subsequent efficacy in controlling lettuce drop caused by *S. minor*, which have higher reported scletorial densities in soil compared to *S. sclerotorium*. Exposure of *C. minitans* spores to UV light (254nm) revealed a negative effect on spore survival (<20% germination after 10 min). Exposure of *C. minitans* to heat also revealed a rapid decline in spore germination after 8 h exposure to high water or soil temperatures (<30% germination after 8 h at 40°C). In field-based experiments, increased application rates of commercial formulation of *C. minitans* - CONTANS^{WG} improved control of lettuce drop caused by *S. minor*. Further studies revealed that one application of CONTANS^{WG} through the sprinkler system (biogation) resulted in a 25% decrease in the incidence of disease, compared to either 8lb/acre CONTANS^{WG} or fungicide (Endura) treatments. Results suggest that application of product via biogation may be a novel strategy for enhanced performance of *C. minitans* in the biocontrol of lettuce drop caused by *S. minor*.

Evaluating the resistance of plantain and banana genotypes to black sigatoka (*Mycosphaerella fijiensis* m.) under greenhouse conditions

E. ALVAREZ (1), A. Cuellar (2)
(1) CIAT, Cali, COLOMBIA; (2) CIAT, Palmira, COLOMBIA
Phytopathology 100:S5

We evaluated, under greenhouse conditions, the reactions of plantain and banana genotypes to the sigatoka fungus, *Mycosphaerella fijiensis*. To inoculate plantain and banana seedlings of different genotypes, we used aqueous suspensions of 5000 conidia mL^{-1} of each of 50 monosporic isolates that had different pathogenicity levels and represented different production areas in Colombia. Disease response according to genotype was assessed by measuring the variables incubation period (IP), time of evolution of symptoms (TES), area under the disease progress curve (AUDPC), and rate of disease development (r). Isolates inoculated on cultivar 'Dominico Harton' demonstrated five levels of pathogenicity (very high, high, medium, low, and very low), which had no relationship with their geographical origin or *Musa* genotype. Isolates of different pathogenicity levels were present in the same zone and for the same genotype. Plantain and banana genotypes reacted differentially to *M. fijiensis* isolates, indicating the possible existence of physiological races in the fungus. Disease severity was classified according to three levels of reactions in *Musa*—resistant, intermediate, and susceptible—where genotypes Topocho, Maqueño, FHIA 20, and FHIA 21 (plantain); and Sedita and FHIA 23 (banana) presented the highest levels of resistance, that is, disease was less severe and slower to progress.

Development of a Real-time PCR assay, to detect and quantify a 16SrIII-L phytoplasma associated with cassava frogskin disease (CFSD)

E. ALVAREZ (1), J. F. Mejia (2), J. M. Pardo (2)
(1) CIAT, Cali, COLOMBIA; (2) CIAT, Palmira, COLOMBIA
Phytopathology 100:S5

Cassava is a major source of carbohydrate in the tropics. It feeds millions of people in America, Asia, and Africa. Yield losses of this root crop to CFSD in Colombia and Latin America can be as high as 90%. Recently, this disease was discovered to be associated with infection by a 16SrIII-L phytoplasma. The disease is exponentially propagated through asexual seed, creating a demand for disease-free planting materials. A TaqMan® probe was designed for the microorganism, based on the *rp* gene (16Sr III-L phytoplasma). We used qPCR to obtain a sensitivity that was 100- and 1,000-fold higher than that obtained from nested. Detection levels were as sensitive as 4×10^2 copies (to obtain the number of copies, we used relative quantification, using an external standard). To validate the technique, we used field-harvested roots showing typical disease symptoms, and obtained an average of 2.32×10^5 copies of the 16Sr III-L phytoplasma. A histological study of different tissues in 3-month-old cassava plants showed that the best tissue for detecting the microorganism is the pith in stems. With this technique, seed can be certified as free of disease, the distribution of the associated microorganism in cassava plants can be ascertained, and the potential for vector insect of the disease discovered.

Sensitivity of *Botrytis cinerea* isolates from strawberry to thiophanate-methyl and iprodione in Michoacan, Mexico

A. Alvarez-Medina (1), A. REBOLLAR-ALVITER (2), H. Silva-Rojas (3)
(1) Universidad Autonoma Chapingo, Morelia, MEXICO; (2) Univ Autonoma Chapingo, Morelia, Mich, MEXICO; (3) Programa de Semillas, Colegio se Postgraduados, Texoco, MEXICO
Phytopathology 100:S6

Grey mold caused by *Botrytis cinerea* is one of the most important pathogens of strawberry in Mexico. Benzimidazole and dicarboxamide fungicides are commonly used for controlling the disease during different times of the growing season. The objective of this research was to determine the sensitivity of several isolates of *B. cinerea* obtained from Zamora (ZV) and Maravatio Valleys (MV), two of the most important regions producing strawberry in Michoacan México, to the fungicides iprodione and thiophanate-methyl. Sensitivity assays were conducted on PDA media amended with fungicide concentrations ranging from 0.01 to 2000 µg/ml for tiophanate-methyl, and from 0.1 to 50 µg/ml for iprodione. Each fungicide was tested against conidia germination and mycelial growth. For iprodione, ED50 values for mycelial growth ranged from 0.23 to 0.89 µg/ml in MV, and from 0.22 to 3.0 µg/ml in ZV. For conidia germination ED50 values ranged from 0.47 to 16.8 µg/ml in MV, and 0.59 to 22.6 µg/ml in ZV. For tiophanate-methyl, ED50 values for mycelia in MV ranged from 0.11 to >2000 µg/ml, and 0.83 to > 2000 µg/ml for ZV. ED50 values for conidia germination in this fungicide ranged from 0.04 to 3.3 µg/ml in MV, and from 0.97 to 3.53 µg/ml in ZV. Overall, ED50 values for both fungicides were higher in Zamora than Maravatio Valley.

UP-PCR analysis and UP-PCR cross-blot hybridization for grouping of Rhizoctonia species isolated from turfgrass in Maryland and Virginia

B. S. AMARADASA (1), B. Horvath (2), D. K. Lakshman (3)
(1) Virginia Tech, Blacksburg, VA, U.S.A.; (2) University of Tennessee, Knoxville, Knoxville, TN, U.S.A.; (3) USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S6

Rhizoctonia brown patch is a serious disease of many turfgrass species in warm regions of the U.S.A. Though hyphal anastomosis reactions have been used to group *Rhizoctonia* species, it is time consuming and sometimes difficult to interpret. Universally primed PCR (UP-PCR) analysis and UP-PCR cross hybridization assay was used for grouping of genetically related isolates. More than 400 *Rhizoctonia* isolates were collected from diseased turfgrass leaves from five geographic areas in Virginia and Maryland. A random sample of 54 isolates was selected and their anastomosis groups (AGs) were determined by hyphal fusion reactions. The isolates were amplified with a fluorescent UP primer to generate multiple PCR fragments for each isolate. The fragment patterns were captured digitally and a dendrogram was constructed. The cladistic analysis supported seven clades including 3 clades of *R. solani* (AG1-1B, AG2-2IIIB and AG5), 1 clade of binuleate Rhizoctonia like fungi (RLF) and 3 clades of *Waitea circinata* (varieties *zeae*, *oryzae* and *circinata*). The UP-PCR analysis corresponded well with traditional AGs by grouping isolates of similar AGs together. The UP-PCR products were also spotted onto a nylon membrane, immobilized and used for cross hybridization against PCR products from tester strains. Genetically related isolates belonging to same AG subgroups cross hybridized strongly while isolates of different AGs gave weak or no signal.

Relationship between pathogenicity and toxin production in Tangerine pathotype of *Alternaria alternata* the causal agent of citrus brown spot in Iran

N. AMINI (1), N. Kakvan (1), H. Zamanizadeh (1), S. Hajmansoor (1)
(1) Science and Research Branch, Islamic Azad University, Tehran, IRAN
Phytopathology 100:S6

Alternaria brown spot is one of the most important diseases of citrus (Tangerine hybrids) all through the world. The disease is caused by *Alternaria alternata* (Fr.) Keissl and makes serious economical losses. This pathogen produces host specific toxins (HSTs) with the same host specificity as the fungal isolates. In the present work, the pathogenic variability of the pathogen was investigated. Furthermore, the possible relationship between the pathogenicity and the ability to produce toxin were investigated for Iranian isolates. A total of 40 isolates of *Alternaria alternata* from Tangerine hybrids were collected from different regions of Iran. Pathogenic variability was evaluated through *in vitro* conditions. The results revealed considerable variation in aggressiveness of the isolates. Cluster analysis of the isolates classified them into two categories: highly and moderate virulent groups. The ability of each isolate to produce toxin was determined by measurement of electrolyte loss from leaf disks. The results showed that culture filtrate dilutions of pathogen were toxic to the natural host under experimental condition. The pathogenicity of *Alternaria alternata* appears to be related to the amount of toxin for each of the isolates tested. This method will hopefully help us to produce more resistant Tangerine hybrids in Iranian citrus breeding programs.

Detection of *Candidatus* Liberibacter asiaticus associated with huanglongbing disease in the salivary glands and alimentary canal of *Diaphorina citri*

E. AMMAR (1), R. G. Shatters (1), D. G. Hall (1)
(1) ARS-USDA, Fort Pierce, FL, U.S.A.
Phytopathology 100:S6

Candidatus Liberibacter asiaticus has been strongly implicated as the causative agent of huanglongbing (HLB), or citrus greening, which is the most devastating citrus disease in Florida and other parts of the world. HLB is transmitted in a persistent manner by psyllid vectors and in the U.S. and Asia by the Asian citrus psyllid *Diaphorina citri.* We used quantitative polymerase chain reaction (Q-PCR) to detect *Ca*. L. asiaticus in dissected organs of individual *D. citri* adult males and females collected from HLB-infected citrus trees in Florida between August and December 2009. The mean proportion of PCR-positive organs was 13–24% for the alimentary canals, 12–16% for the salivary glands, and 16–25% for the rest of the insect body. Percentage of infection did not differ significantly between the three insect parts in males but was significantly lower in the salivary glands than in the alimentary canals of females. Our results provide the first PCR confirmation of *Ca.* L. asiaticus in the alimentary canal and salivary glands of *D. citri*, and suggest that both organs may constitute major transmission barriers to this bacterium in the psyllid vector. We are currently testing other techniques including TEM, immunolabeling, and *in situ* hybridization for the purpose of elucidating the transmission barriers and cellular interactions of *Ca.* L. asiaticus in this economically important vector.

Detection of *Xylella fastidiosa* in petioles is independent of sample storage time and temperature

B. F. AMSDEN (1), P. Vincelli (1), J. R. Hartman (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S6

Leaf petioles collected for isolation of *Xylella fastidiosa* are usually processed within 12 hours of collection to optimize culturing the fastidious bacterium. However, leaf samples collected from species of shade trees, horticultural shrubs, and grape vines showing symptoms of bacterial leaf scorch are often sent to our Diagnostic Laboratory several days after being collected. ELISA-positive samples with weak to moderate ELISA scores will sometimes yield a weak positive reaction by PCR, suggesting the possibility that sample handling conditions may have detrimental effects on detection of *X. fastidiosa*. Our sample storage study suggests that neither storage temperature (ambient room temperature, 4°C, –20°C, or –80°C) nor duration of storage (≤24 hours or 6 days) affects the ability to detect *Xylella fastidiosa* by real-time PCR in petioles of shade trees, shrubs, and grape. The use of ELISA sample extract as a source of *X. fastidiosa* DNA reduced the amount of time and effort required to conduct PCR detection in bacterial leaf scorch suspects, compared to TE bacterial release (pulverized infected tissue resuspended in TE buffer and used directly in PCR reactions without DNA extraction) or total DNA purification by QIAamp DNA Stool Kit methods.

Sporulation of *Phomopsis viticola* on infected grape tissues

D. J. ANCO (1), L. V. Madden (1), M. A. Ellis (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S6

Phomopsis viticola is the causal agent of Phomopsis cane and leaf spot on *Vitis* spp. (grapes), which is a serious and economically important disease in temperate regions. This disease is currently understood to be monocyclic, with primary inoculum only being produced early in the growing season. The objective of this study was to determine if secondary inoculum can be produced from primary infections on internodes and rachises during the growing season. Infected first-year canes and rachises were collected throughout the growing season of 2009 and observed for production of pycnidia and sporulation after 48 h incubation in a mist chamber. Tissues were collected from 'Catawba,' 'Concord,' and 'Reliance' vineyards and washed with deionized water, and conidia were counted from these washings with a hemacytometer. In 2009, the potential for lesions on infected grape canes to produce conidia was not observed until after harvest. Very low amounts (e.g., 1.5 alpha-conidia/mm^2) of conidia were observed on some rachises during the growing season; however, we were unable to isolate *P. viticola* from these conidial suspensions. These results confirm that Phomopsis cane and leaf spot is a monocyclic disease.

Effects of temperature and wetness duration on the sporulation rate of *Phomopsis viticola* on infected grape canes

D. J. ANCO (1), L. V. Madden (1), M. A. Ellis (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S6

Phomopsis viticola is the causal agent of Phomopsis cane and leaf spot on *Vitis* spp., a serious and economically important disease of grapes in

temperate regions. This disease is currently understood to be monocyclic, with inoculum being produced early in the growing season. The objective of this study was to examine the effects of temperature and wetness duration on the sporulation rate of *P. viticola* in order to develop a predictive model for sporulation in the vineyard. Infected first-year 'Catawba' canes were collected in January, 2009, and stored at –20°C. Canes were placed in mist chambers and incubated under various combinations of temperature and wetness duration. Experimental design was a split-plot, with temperature (5, 12, 15, 18, 20, 22, 25, 28, and 35°C) as the main-plot and wetness duration (11, 23, 35, 47, and 71 h) as the sub-plot. Following incubation, canes were washed with deionized water, and alpha-conidia were counted with a hemacytometer. Results from 2009 indicate that sporulation of *P. viticola* on infected canes can occur between 5 and 35°C, and is optimal near 22°C. Little to no sporulation was observed after 11 h wetness duration from 5–35°C; sporulation increased with increasing wetness duration at each temperature.

Regulatory role of c-di-GMP biosynthesis genes of *Xylella fastidiosa*'s virulence factors

V. ANCONA (1), D. N. Appel (1), P. de Figueiredo (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S7

Xylella fastidosa (*Xf*) is a bacterial plant pathogen that has been recognized as the causing agent of several plant diseases including Pierce's disease of grapevine (PD). Currently there are no commercial resistant varieties of grape or an effective method for controlling PD. Therefore, this disease threatens the U.S. grape and wine industries. Although *Xf* is known for causing PD, the regulatory mechanisms that mediate virulence in the pathogen remain unclear. Illuminating the molecular mechanisms mediating biofilm formation and expression of virulence factors will promote the development of strategies for controlling PD. Our project focuses on characterizing the role that cyclic diguanylate (c-di-GMP) metabolic proteins play in regulating biofilm formation, cell aggregation and virulence of *Xf*. c-di-GMP is a second messenger that regulates aggregation, biofilm formation, and virulence in several bacterial pathogens. This molecule is synthesized by diguanylate cyclase enzymes (DGCs) and is degraded by phosphodiesterases (PDE). DGC and PDE activities reside in the GGDEF, EAL or HD-GYP domains of proteins respectively. A diverse array of bacteria harbor genes that encode these enzymes. In fact, 5 genes have been identified in the *Xf* genome that are predicted to encode proteins containing the conserved GGDEF, EAL and/or HD-GYP domains. Mutations in these genes result in altered biofilm and aggregation phenotypes suggesting a direct regulatory role on the expression of *Xf* pathogenicity traits.

Biochemical and microscopical study of *Phytophthora infestans* process of infection on *Physalis peruviana*

C. A. ANTOLÍNEZ (1), G. Danies (1), G. Peña (1), Á. M. Vargas (1), A. J. Bernal (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA
Phytopathology 100:S7

Phytophthora infestans is a plant pathogen that affects a great variety of crops within the Solanaceae family. The pathogen has been described causing disease in potato, tomato, lulo, tree tomato and in 2007 it was described causing disease in *Physalis peruviana* (cape gooseberry). Since the report of the disease, we have studied this particular interaction and we believe that the infection process of cape gooseberry is different than in potatoes. The aim of this work was to characterize the first defense reactions produced on cape gooseberry leaves due to the infection with *P. infestans*. Detached Cape gooseberry leaves were inoculated with a solution of 10^4 sporangia/ml. Electron microscope photographs were taken at 0, 24, 48, 72 and 96 hours post inoculation on the leaf abaxial surface up. Reactive oxygen species (ROS) and induction of pathogen related proteins were measured at 0, 6, 12, 18, 24, 48 y 72 hours after inoculation and 6, 9, 12, 15 and 18 days after inoculation. *Phythopthora* germinated and showed active aerial growing but no evidence of penetration was found in the first days after the infection. It seems that the cape goosberry ecotype used in this study is resistant to *P. infestans*. To our knowledge this is the first study that characterizes the first biochemical reactions caused by *P. infestans* on cape gooseberry. Our results are fundamental for understanding how *P. infestans* affects other members of the Solanaceae family.

PCR detection and identification of *Phymatotrichopsis omnivora*

M. ARIF (1), S. M. Marek (1), F. M. Ochoa Corona (1), C. Young (2), C. D. Garzon (1)
(1) Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, U.S.A.; (2) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 100:S7

The soilborne pathogen, *Phymatotrichopsis omnivora* is the causal fungus of cotton root rot of numerous dicots in the southwestern United States and Mexico. Disease diagnosis depends on the presence of characteristic mycelial cords on the roots of wilted plants, which are not always obvious. Early, accurate and sensitive detection of *P. omnivora* in affected plant tissues is needed by plant health officials for inspection of products from quarantined states, and locally, by extension specialists to predict disease outbreaks and identify reservoir hosts. Specific PCR primers recognizing conserved rDNA-ITS sequences were designed based on an alignment of 144 sequences from *P. omnivora* isolates collected throughout North America. Three primer pairs, PO1 (415 bp product), PO2 (499 bp product) and PO3 (146 bp product), 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, asymptomatic plants. PO1, PO2 and PO3 detected 5×10^{-7}, 5×10^{-8} and 5×10^{-8} ng per µl of the *P. omnivora* target DNA, respectively. PO3 also was compatible with qPCR and thermostable helicase dependent amplification (tHDA) assays. The described PCR assays should be useful for rapid, sensitive diagnosis, quantification of infection in resistance breeding programs and agricultural biosecurity.

Highly sensitive molecular detection of five *Pythium* species

M. ARIF (1), F. Flores (1), F. M. Ochoa-Corona (1), C. Garzon (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S7

Detection and discrimination of *Pythium* species, the causal agents of root rot and damping-off of seedlings, is difficult if based only on morphological characteristics. Five sets of specific primers were designed from consensus rDNA-ITS sequences of *Pythium aphanidermatum*, *P. deliense*, *P. spinosum*, *P. irregulare* and *P. cryptoirregulare* retrieved from Genbank. The sequences were aligned using ClustalX2 and were edited manually. Primer design was accomplished using Web interface software Primer3, mFOLD and BLASTn with validated thermodynamic parameters. Primers sets were validated *in silico* against published sequences of the five species of *Pythium*, and *in vitro* against isolates of the target species and related species. The amplified PCR products were cloned and sequenced. Extensive PCR assays were conducted to determine the sensitivity of each set of primers and optimal annealing temperatures. The sensitivity of the primers for detection of *P. aphanidermatum*, *P. deliense*, *P. spinosum*, *P. irregulare* and *P. cryptoirregulare* is of 10^{-5}, 10^{-2}, 10^{-6}, 10^{-4} and 10^{-6} ng, respectively. This set of PCR assays allows the rapid detection of, and discrimination among, five *Pythium* species for applications in agricultural biosecurity and microbial forensics.

Increased PCR amplification incorporating primer flap sequences and free energy values near equilibrium

M. ARIF (1), I. Oikonomakos (1), D. Caasi (1), F. M. Ochoa-Corona (1)
(1) Oklahoma State University, Department of Entomology and Plant Pathology, National Institute for Microbial Forensic and Food & Agricultural Biosecurity (NIMFFAB), Stillwater, OK, U.S.A.
Phytopathology 100:S7

Successful PCR amplifications rely on precise design of oligonucleotide primers. Primer design still poses unresolved questions, particularly in studies in which real time and/or end point PCR assays are resolved with SYBR green. The aim of this study is to understand the effect on PCR yield of using primers with minimal free energy (ΔG) and 5' AT-rich overhanging sequences. Specific primers for *Pythium cryptoirregulare* with ΔG=0 and a second pair with ΔG=1 were designed based on consensus rDNA-ITS sequences retrieved from Genbank. The sequences were aligned using ClustalX2 and manually edited. Both set of primers were designed using validated thermodynamic parameters and Web interface software Primer3, mFOLD and BLASTn. Primers were validated *in silico* against published *P. cryptoirregulare* sequences and the amplified products were cloned and sequenced. No primer dimer formation was observed in PCR amplifications using primers with ΔG=0. Dimer formation and SYBR green misleading signals were observed with primers having ΔG=1 in real time PCR assays. Primers with both minimal ΔG value and 5' AT-rich overhangs showed enhanced total PCR yields and accuracy. The described primer design parameters have the potential for improving the efficiency of existing PCR based assays and increasing the detection sensitivity of predetermined targets in agricultural biosecurity and microbial forensics.

Expression profiling of host response of citrus to Candidatus Liberibacter asiaticus infection

V. ARITUA (1), N. Wang (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S7

Three species of Candidatus Liberibacter: C. Liberibacter asiaticus (Las), C. Liberibacter africanus and C. Liberibacter americanus are associated with

Huanglongbing disease of citrus. Of the three phloem-limited α-proteobacteria, Las has a worldwide distribution in citrus producing areas including U.S. (Florida and Louisiana). A recent study indicates that Las infection results into significant differences in response between citrus varieties or relatives although none has been found to be resistant. Systemic infection studies also show un-even distribution patterns of Las populations among different organs and tissues of citrus. These results suggest that cellular response to Las is genotype dependent and may be tissue specific as well. Since, the molecular basis of the interaction is not yet fully elucidated, we are conducting transcriptional analyses of citrus varieties with varying degrees of response to Las infection at different infection stages in greenhouse and citrus grove. We hypothesize that Las modulates different cellular processes in citrus in time and space. Preliminary analysis of RNA from Las-infected Valencia sweet orange (Citrus sinensis) roots using suppression subtractive hybridization showed up-regulation of pathogenesis/resistance, biotic stress related and cell wall re-modeling gene groups and transcriptional factors. Some disease resistance genes homologous to nucleotide binding and Leucine-rich repeat (NB-LRR) domain containing class of proteins were down regulated.

A bioinformatic study of pathogenicity factors in *Xanthomonas axonopodis* pv. *manihotis*

M. L. ARRIETA (1), L. M. Rodriguez-R (1), R. Koebnik (2), S. Restrepo (1), A. Bernal (1)
(1) Universidad de los Andes, Bogotá, COLOMBIA; (2) Institut de recherche pour le développement, Montpellier, FRANCE
Phytopathology 100:S8

Xanthomonas axonopodis pv. *manihotis* (Xam) is a gram-negative bacterium and the causal agent of Cassava Bacterial Bligth. Information about pathogenicity determinants in this bacterium is currently limited in the literature. The aim of this study was to increase the understanding of this plant-pathogen interaction using genomics. A draft genome sequence of Xam strain CIO151 was produced using second generation sequencing technology and gene prediction was performed on the partial sequence. Based on similarity analyses, putative protein annotation was performed. The G+C content of the partial sequence (64.6%) was similar to that reported for other species of *Xanthomonas*. Nevertheless, a significant part of genome sequence (12.25%) showed an atypical G+C content, a fact that could suggest horizontal gene transfer events. We report a preliminary set of effector sequences in Xam, as well as the presence of sequences with similarity to genes involved in regulation of pathogenicity factors (*Rpf*) and Xanthan biosynthesis (*gum*). Also, putative proteins that could be involved in pathogenicity, such as the ones involved in assembly of protein Secretion Systems (Type I through III) were found. The partial genomic data here suggest that Xam shares many pathogenicity factors with closely related bacteria in the genus Xanthomonas. On the other hand, several changes in the analyzed genes could account for host specificity differences with other bacteria in the species *X. axonopodis*.

Effect of leaf age on primary infection and development of colonies of strawberry powdery mildew

B. ASALF (1), A. Stensvand (2), D. M. Gadoury (3), R. C. Seem (3), A. Tronsmo (2)
(1) Norwegian University of Life Sciences, Aas, NORWAY; (2) Aas, NORWAY; (3) Geneva, NY, U.S.A.
Phytopathology 100:S8

Development of ontogenic resistance to powdery mildew (*Podosphaera aphanis*) on strawberry leaves has been reported, however, the components of resistance have not been elucidated. Five developmental stages of strawberry leaves were identified and assigned numerical values from newly emerged and unexpanded (S1) to fully expanded and dark green (S5) of cvs. Korona and Senga Sengana. The upper and lower surface of the leaves were inoculated from each of the five leaf developmental stages and incubated under controlled conditions. The effect of leaf age on germination, infection efficiency, latency period, and sporulation were later evaluated. All responses were significantly ($p = 0.05$) affected by leaf age. Germination percentage, infection efficiency, and sporulation were highest, and latent periods were shortest on S1 leaves of both cultivars. On Senga Sengana, germinating conidia produced fewer secondary hyphae during infection. Conidia produced very few secondary hyphae and did not sporulate on S3 leaves, and no infections established on S4 or S5 leaves. The high success of infection and colonization of *P. aphanis* on S1 leaves indicates that disease is established preferentially on emergent and expanding leaves and these should be the target of management strategies.

Spinach seed a source of *Verticillium dahliae* in lettuce in coastal California

Z. K. ATALLAH (1), K. Maruthachalam (1), K. V. Subbarao (1)
(1) University of California-Davis, Salinas, CA, U.S.A.
Phytopathology 100:S8

Fungal migrations in infested seed or vegetative material are cause for concern and regulation. To clarify the mechanisms that led to the recurrent epidemics of Verticillium wilt on lettuce in coastal California, we used 22 simple sequence repeat markers to retrace the evolutionary and migratory histories of *Verticillium dahliae*. The markers were used on strains isolated from: lettuce and other vegetable and small fruit plants growing coastal California; tomato and lettuce seed from two inland California valleys; spinach seed from northern Europe, Washington State and Chile; and ornamentals from Wisconsin. There was no differentiation between the spinach seed sub-populations and the other sub-populations, with the exception of tomato, suggesting gene flow. Migration analyses also suggested this gene flow from spinach seed sources. Haplotypes from coastal California lettuce were assigned to spinach sub-populations, and similarly many spinach seed haplotypes were assigned to non-self spinach sources. However, no strains were assigned to the lettuce seed sub-population, including those isolated from lettuce plants growing 60 Km away. Similarly, the structure of the coastal California lettuce plant sub-population was similar to spinach seed sub-populations, but not to other California sub-populations. This evidence of migration corroborates the proposition that massive *V. dahliae* invasions in germplasm shape population structures effectively regardless of the carrier host germplasm.

Mitigation of aflatoxin contamination in Nigerian maize with atoxigenic strain mixtures

J. ATEHNKENG (1), P. J. Cotty (2), R. Bandyopadhyay (1)
(1) International Institute for Tropical Agriculture, Ibadan, NIGERIA; (2) University of Arizona, USDA ARS, Tucson, AZ, U.S.A.
Phytopathology 100:S8

In West Africa, aflatoxin contamination of maize is a frequent perennial problem resulting in serious impacts on health and trade. Competitive exclusion of aflatoxin producers by atoxigenic strains of *Aspergillus flavus* is a viable option for aflatoxin management. We evaluated the field efficacy of a mixture of four atoxigenic strains in reducing aflatoxin contamination through displacement of aflatoxin producers in maize during the 2007 and 2008 seasons in four states of Nigeria. Sterile sorghum grains were colonized independently by each of four atoxigenic strains. After drying, colonized grain was mixed so that the four strains were in equal proportions. The mixture was broadcast over maize crops at 10 kg/ha 2–3 weeks before flowering. Grain from treated and control fields were analysed for aflatoxins both at harvest and after storage. In both years, the atoxigenic strain applications reduced both maize contamination and the proportion of the crop infecting *A. flavus* population composed of aflatoxin producers. Aflatoxin reductions of 67% to 95% were associated with 74% to 80% displacement of aflatoxin producers. The results demonstrate that effective atoxigenic strains native to West Africa can be selected from fungal communities associated with maize production and successfully utilized in programs to mitigate aflatoxin exposure in human populations.

A flgB mutation in *Xanthomonas axonopodis* pv. *glycines* confers reduced bacterial pustule disease of soybean

D. ATHINUWAT (1), T. J. Burr (1), S. Prathuangwong (2)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Kasetsart University, Bangkok, THAILAND
Phytopathology 100:S8

Xanthomonas axonopodis pv. *glycines* KU-P-SW005, the cause of bacterial pustule disease on soybean, has a single monopolar flagellum that is associated with swimming motility, biofilm formation, and virulence on soybean. A targeted mutation in flgB, encode a flagella basal body rod protein was generated with an EZ::TN transposome system. The flgB mutant lacked flagellum and was swimming-minus. Furthermore, it generated an altered biofilm (less robust than wildtype) and caused reduced disease severity on soybean. Following inoculation of cv. Spencer, the flgB mutant gave a virulence rating of 9.89% as compared to 51.98% for wildtype at 10 days post inoculation. Complemented flgB mutant restored swimming motility, biofilm and disease equal to the wildtype.

Control of peanut rust with fungicides in Nicaragua

J. AUGUSTO (1), T. B. Brenneman (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S8

Peanut rust (caused by *Puccinia arachidis*) is an important disease that limits peanut production in Nicaragua. Several fungicide programs were evaluated from 2005 to 2009 in randomized block designs at six locations planted with cv. Georgia Green. Four applications of tebuconazole formulations at 0.23 kg a.i./ha (Tacora 25 EW, Folicur 25 EW, Orius 25 EW, and Tebuconazole 25%) and chlorothalonil at 0.96 kg a.i./ha (Chlorothalonil 720) did not differ in rust control, and all were effective in reducing disease severity. Tebuconazole formulations had similar yields but higher than chlorothalonil. Pyraclostrobin + epoxiconazole (Opera, 0.14 kg a.i./ha), tebuconazole (Tebuconazole 25%),

epoxiconazole + carbendazim (Duett, 0.18 kg a.i./ha), azoxystrobin + cyproconazole (Amistar Xtra, 0.10 kg a.i./ha), and chlorothalonil applied twice either at night (3 to 5 a.m., when leaves were folded) or during the day (10 a.m. to 12 p.m., when leaves were unfolded) all decreased rust severity compared with the control, and application timing were not different. Night fungicide applications generally had higher yields than day applications presumably due to improved control of stem rot (*Sclerotium rolfsii*). Applying pyraclostrobin + epoxiconazole, azoxystrobin + cyproconazole, picoxystrobin (Acapela 25 SC, 0.10 kg a.i./ha), and trifloxystrobin + propiconazole (Stratego, 0.16 kg a.i./ha) at applications 2 and 3, vs. 3 and 4, vs. 2 and 4 gave similar rust control. Opera was generally the most effective fungicide for rust control and yield increase.

Molecular characterization of resistance to boscalid and penthiopyrad in *Didymella bryoniae* isolates collected from Georgia watermelon fields

H. F. AVENOT (1), A. Thomas (1), R. D. Gitaitis (1), D. B. Langston (1), K. L. Stevenson (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S9

In order to elucidate the genetic basis of resistance to the succinate-dehydrogenase-inhibiting fungicides (SDHIs) boscalid and penthiopyrad in field isolates of the gummy stem blight pathogen *Didymella bryoniae*, a 506-bp fragment of the iron sulphur gene (*DbSDHB*) was amplified from a fungicide-sensitive isolate of *D. bryoniae*. The deduced amino-acid sequence showed high similarity with iron sulphur proteins (Ip) from other organisms. A primer pair was designed from the obtained sequence and used to specifically amplify the region containing the highly conserved histidine residue known to confer resistance to SDHIs in several fungal species. Subsequent comparison of the corresponding sequences of *DbSDHB* from sensitive and resistant isolates revealed that the precise histidine residue (codon CAC) was present in 12 sensitive isolates, but in 73 boscalid- and penthiopyrad-resistant isolates the histidine codon was converted to tyrosine (codon TAC). In 7 other isolates that were found to be resistant to boscalid, but unpredictably sensitive to penthiopyrad, the histidine residue was replaced by arginine (codon CGC). These findings seem to indicate that while the histidine-tyrosine mutation conferred resistance to both fungicides, the replacement of histidine by arginine conferred resistance to boscalid, but had no impact on penthiopyrad sensitivity. The polymorphisms thus revealed in *DbSDHB* sequences will be used to develop rapid molecular diagnostic assays to detect SDHI resistance in this pathogen.

Validating environmental parameters for primary infection of grapes by *Erysiphe necator* ascospores under Michigan conditions

L. L. AVILA (1), A. R. Sullenger (1), J. Kroll (2), N. L. Rothwell (2), A. M. C. Schilder (1)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) Michigan State University, Traverse City, MI, U.S.A.
Phytopathology 100:S9

Powdery mildew, caused by *Erysiphe necator*, is a common and widespread disease of grapevines. In the spring, the fungus releases ascospores from overwintering cleistothecia after 2.5 mm of rain and temperatures of 10°C or above. To improve disease development predictions, ascospore release and primary infection were monitored in unsprayed areas of research vineyards in Clarksville and Traverse City, MI. Campbell weather stations were used to monitor environmental conditions at the sites. Burkard spore traps (both vineyards) and potted cv. Chardonnay bait plants (Clarksville only) were placed between vines from before bud break until fruit set. Burkard reels and plants were changed weekly and ascospores and powdery mildew colonies counted. Ascospores were detected in the air for more than a week after rain events from May until the end of June. Peak ascospore release occurred in Clarksville on May 22–26 and in Traverse City on June 9, 2009. The presence of powdery mildew colonies on bait plants also indicated ascospore activity since early May. However, infections of field-grown vines did not become evident until late June in Clarksville and late August in Traverse City, indicating a discrepancy between the presence of primary inoculum and disease development. This may be related to the exceptionally cool and rainy 2009 growing season. Further investigation is needed to better predict powdery mildew development under Michigan weather conditions.

Screening for strobilurin (QoI) resistance in grape powdery mildew populations in Michigan

L. L. AVILA (1), T. D. Miles (1), W. W. Kirk (1), A. M. C. Schilder (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S9

Powdery mildew, caused by *Erysiphe necator*, is the most common and destructive disease of grapes worldwide. In Michigan, it is primarily controlled with fungicides, including strobilurins (Quinone outside Inhibitors [QoIs]). Resistance to this class of fungicides has been reported in *E. necator* in New York and Virginia. To determine whether QoI resistance occurs in Michigan, 12 *E. necator* isolates were collected from 5 vineyards in 2008 and tested by PCR for the G143A single-nucleotide mutation responsible for QoI resistance. The mutation was detected in one isolate, which was confirmed to be resistant in a conidium germination assay on water agar amended with trifloxystrobin at 0, 0.001, 0.01, 0.1, 1, 10, or 100 µg/mL and salicylhydroxamic acid (100 µg/mL). The mutant was able to germinate at 100 µg/mL, whereas a representative wildtype isolate did not germinate beyond 0.01 µg/mL. In 2009, 173 isolates were collected from 2 vineyards with no fungicide application history, and 14 commercial and 6 research vineyards. Isolates were screened for QoI resistance as described above. Isolates in unsprayed vineyards had EC_{50} values mostly below 0.01 µg/mL, while isolates that were highly resistant to trifloxystrobin (EC_{50}>100 µg/mL) occurred in 4 commercial and 5 research vineyards at frequencies of 11–50% and 50–100%, respectively. These results suggest that fungicide resistance may play a role in poor control of powdery mildew observed in some Michigan vineyards.

Effects of extracts of some plants on the wet rot of *Amaranthus cruentus* L. induced by *Choanephora cucurbitarum* and on the performance of the crop

A. N. AWURUM (1)
(1) Michael Okpara Univ of Agriculture, Umdike, Umuahia, Abia State, NIGERIA
Phytopathology 100:S9

Amaranthus is one of the important leafy vegetable crops in Nigeria. The crop sustains severe losses due to wet rot induced by *Choanephora cucurbitarum*. Limitations in the use of synthetic chemicals in the control of horticultural crop diseases continuously increase due to their negative environmental impact. This has shifted attention to safer materials. The evaluation of the efficacy of leaf extracts of *Dennettia tripetala*, *Spondias mombin* and *Bryophyllum pinnatum* in controlling the growth of *Choanephora cucurbitarum* was carried out in the greenhouse. The experiment was a 3 × 5 factorial in a completely randomized design (CRD) replicated 5 times. The treatments comprised 3 methods of inoculation of the pathogen; un-inoculated seeds, inoculated seeds and inoculated plants at 6 weeks. The chemicals were benomyl a synthetic fungicide, extracts from different plants and sterile water as control. From the result of the experiment *D. tripetala* significantly ($P < 0.05$) reduced the severity of the wet rot induced by *C. cucurbitarum* more than the other plant extracts having a severity score of 5.8 in a 10 – point scale, but the effect was not significantly ($P < 0.05$) different from that of benomyl. Among the plant extracts, *B. pinnatum* was the least effective with a severity score of 8.0. Plants in the control pot had the highest severity score of 9.8. The plants treated with benomyl and the plant extracts grew better and had significantly higher total plant dry matter than plants in the control pots.

Downy mildew of basil in Illinois

M. BABADOOST (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S9

Downy mildew of basil, caused by *Peronospora belbahrii*, first occurred in Illinois in 2009. The disease was detected in commercial fields throughout the state. The first report of the disease in the United States was from Florida in 2007. *P. belbahrii* infects leaves, rapidly develops and spreads, and can cause total crop loss. A trial was conducted in a commercial basil field at Momence, Illinois, during September-October 2009 to evaluate the efficacy of selected fungicides for control of downy mildew of basil. The trial included 12 treatments, which were performed in a randomized complete block design with four replications. The field had been planted in April 2009 and plants were actively growing with moderate infection of downy mildew on leaves. Four spray-applications of the fungicides were made at 7-day intervals and disease severity in the plots was assessed 7 days after the last spray application. Severity of downy mildew was less than 4% in the plots sprayed with chlorothalonil (Bravo Weather Stik), dimethomorph (Forum), zaxomide + mancozeb (Gavel), fluopicolide (Presidio), azoxystrobin (Quadris), cyazofamid (Ranman), mandipropamid (Revus), and famoxadone + cymoxanil (Tanos), when these fungicides were mixed with potassium phosphite (ProPhyt). Severity of the disease was the lowest (1%) in plots sprayed with Quadris; was highest (38.1%) in control plots. Severity of the disease was 18.1% in the plots sprayed with an organic mix.

The Egestion-Salivation Hypothesis: Evidence for the role of vector saliva in the inoculation mechanism of *Xylella fastidiosa*

E. A. BACKUS (1), K. Andrews (2), J. M. Labavitch (3), C. Greve (3)
(1) USDA ARS, Parlier, CA, U.S.A.; (2) Australian Dept. of Primary Industries, Attwood, AUSTRALIA; (3) University of California-Davis, Davis, CA, U.S.A.
Phytopathology 100:S9

Despite ca. 40 years of study, the mechanism of inoculation of the Pierce's Disease bacterium, *Xylella fastidiosa* (*Xf*), by vectors such as the glassy-

winged sharpshooter (GWSS) is still unknown. Research on the Egestion-Salivation Hypothesis for *Xf* inoculation will be presented. Two important steps in this hypothesis are uptake of saliva containing the cell wall-degrading enzyme beta-1,4 glucanase into the precibarium where *Xf* colonies develop, followed by injection of this enzyme-containing saliva into the xylem prior to ingestion. To directly test the role of saliva in inoculation, immunohistology was used to study interactions between *Xf* and GWSS saliva in grapevine. Adult GWSS were confined in small cages on grapevine stems for 24 hours and allowed to probe, leaving salivary deposits in the plant. *Xf* was then needle-inoculated into the same stem area; 1 hour later, the tissue was excised and prepared for immunohistology using a commercial *Xf* probe. *Xf* bacteria observed in xylem cells penetrated the semi-viscous saliva deposited during GWSS probing prior to *Xf* inoculation. Therefore, *Xf* bacteria have the ability to infiltrate gelled saliva containing salivary glucanase. This suggests that, in a natural GWSS inoculation, *Xf* could potentially migrate out of injected saliva and into xylem fluid. Implications for the mechanism of inoculation are discussed.

Production of mycotoxins by members of the *Aspergillus* section *Nigri* isolated from peanuts and maize in the United States

C. Bacon (1), E. PALENCIA (2)
(1) USDA ARS, Athens, GA, U.S.A.; (2) Department of Plant Pathology, University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S10

Fungi of the *Aspergillus* section *Nigri* (black aspergilli) are pathogenic to maize, grapes, onions, garlic, apples, mangoes, and peanuts. Although some black aspergilli are reported as opportunistic pathogens, other species are able to colonize maize seedlings as symptomless endophytes, which under stress, can develop symptoms of seedling blight or later on symptoms similar to *Fusarium* ear rot disease. The main concern for crops infected by black aspergilli is the production of toxic secondary metabolites. Ochratoxin A, the fumonisins, and penicillic acid are examples of these metabolites that are carcinogenic to animals and are thereby classified as mycotoxins. The aim of this research was to screen 60 field black *Aspergillus* strains, isolated as asymptomatic endophytes from peanut and maize, for production of these mycotoxins. HPLC-MS/MS analysis detected the production of ochratoxin A, fumonisin B1, and penicillic acid when strains were cultured on maize, wheat, and rye seeds. Our results indicated that the most dominant species isolated was *A. niger* var. *niger*, and less than 20% of the field isolates were able to produce ochratoxin A, while less than 10% produced fumonisins B1. Pencillic acid was produced in high amounts (> 10 ppm) by these isolates, which is the first report for the production of this mycotoxin by members of the *Nigri* section. The fumonisins and penicillic acid are also phytotoxic and might play roles in diseases of peanuts and maize.

Relationship of substrate and surfactin production by *Bacillus mojavensis* strains and their antagonistical response to *Fusarium verticillioides*

C. W. BACON (1), D. M. Hinton (2), T. Mitchell (2), M. Snook (2)
(1) USDA ARS, Athens, GA, U.S.A.; (2) USDA, ARS, Russell Research Center, Athens, GA, U.S.A.
Phytopathology 100:S10

The endophytic bacterium, *Bacillus mojavensis*, RRC 101 controls fungal diseases in maize and other plants. The bacterium and its cultural extracts have been shown to be antagonistic to the pathogenic and mycotoxic fungus, *Fusarium verticillioides*. An antifungal lipopeptide produced by *B. mojavensis* strains in culture was identified as surfactin, a biosurfactant. HPLC-MS spectra analyses indicated that *B. mojavensis*, RRC 101, produced Leu7-surfactin as the major surfactin, although in the surfactin complex C-14 and C-15 isoforms dominated. Bacterial strains and culture media can have a direct effect on antifungal antagonism, surfactant production, and metabolic utilization by strains of *B. mojavensis*. In this investigation, *B. mojavensis* strains were screened to determine the effects of media on antagonism to *F. verticillioides*, surfactant production, and metabolic utilization of key substrates. The data indicated that the bacterial strains showed zero to high levels of antagonisms, which were not correlated with total surfactin production. Thus, the data suggest that either there are synergistic effects from specific isoforms of surfactins produced on agar media or there are also some unidentified biosurfactants or there areother inhibitory compounds yet determined.

Genetic complementation between two viruses facilitates the systemic movement of a gene silencing suppressor in an otherwise restrictive host

S. BAG (1), N. Mitter (2), H. R. Pappu (1)
(1) Department of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Queensland Agricultural Biotechnology Centre, St. Lucia, Qld 4067, AUSTRALIA
Phytopathology 100:S10

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, TSWV facilitates the systemic movement of only the NSs gene of IYSV, and the systemic symptoms produced by TSWV in the presence of the IYSV silencing suppressor are more severe than those caused by TSWV infection alone. The selective movement of the silencing suppressor gene of one tospovirus species into younger, uninoculated leaves of an otherwise restrictive host suggests complementation between two distinct tospovirus species.

DNA microarray based universal plant virus detection and identification

B. BAGEWADI (1), D. C. Henderson (2), R. L. Jordan (2), K. L. Perry (3), U. Melcher (4), D. Wang (5), K. Fischer (6), J. Hammond (2), C. M. Fauquet (5)
(1) Donald Danforth Plant Science Center, St. Louis, MO, U.S.A.; (2) Beltsville, MD, U.S.A.; (3) Ithaca, NY, U.S.A.; (4) Stillwater, OK, U.S.A.; (5) St. Louis, MO, U.S.A.; (6) Salt Lake City, UT, U.S.A.
Phytopathology 100:S10

To keep pace with the ever increasing number of plant viruses an effective detection and identification system is essential to prevent the introduction and spread of potentially devastating plant epidemics. DNA microarray methods based on oligonucleotide probes offer cheap, rapid, reliable, and parallel detection of plant pathogens including viruses. Previously published studies have focused either on a single crop or family of plant viruses. To prototype the development of taxonomy based DNA microarray diagnostic system for all the plant virus species and sub-viral entities, we selected conserved multiple probes for 54 plant viruses representing 17 virus families/groups and printed on poly-lysine coated glass slides. For both RNA and DNA plant viruses we used 2–5 µg of total RNA extracted from virus infected *N. benthamiana* or host plants to post label with Cy3 fluorescent dye. Slides were scanned using Axon 4000B scanner and spots were quantified using Genepix Pro software. Using the prototype DemoPlantVirusChip a total of 22 plant viruses (e.g., ACMV, TGMV, CMV, PVX, PVY and TMV) were detected over a wide dynamic range with a sensitivity of 5 ng of amino-allyl labeled DNA. As a pilot test, we are now testing this chip with 200 samples from a potato gene bank from CIP and with 140 plant infected samples from ATCC. In the phase II, we are now developing the full version of the plant virus chip with 60-mer probes for every taxon/node of all plant viruses.

RT-qPCR analysis of genes associated with chestnut blight in susceptible American and resistant Chinese chestnut trees

K. BAIER (1), C. A. Maynard (1), W. A. Powell (1)
(1) SUNY ESF, Syracuse, NY, U.S.A.
Phytopathology 100:S10

American chestnut (Castanea dentata) is highly susceptible to infection caused by the necrotrophic fungus, Cryphonectria parasitica. Closely related Chinese chestnut (C. mollissima) exhibits a natural resistance to blight. Genes commonly induced during plant defense response to infection, including genes for β−1,3−glucanase, cinnamyl alcohol dehydrogenase (CAD) and laccase, were preliminarily identified as differentially expressed between Chinese and American chestnut using suppression subtractive hybridization (SSH). Full-length cDNA sequences of these genes have been identified in both chestnut species using the Fagaceae genomic website (http://www.fagaceae.org/home). Genes from the two species are very similar in DNA sequence, therefore it is likely the expression patterns of specific genes is important to conferring the blight-resistance in Chinese chestnut. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to confirm and quantify differential gene expression between American and Chinese chestnut stem tissues. Results indicate there is significantly higher expression of some defense related genes in stems of Chinese chestnut than in American chestnut. Laccase, for example, had several hundred fold higher expression in Chinese chestnut seedlings. Genes more highly expressed in Chinese chestnut would be good candidates for use in genetic modification of American chestnut to determine if they can enhance resistance to chestnut blight.

Evaluation of nitric oxide detoxifying flavohaemoglobin in the *Fusarium verticillioides* – maize interaction

T. T. BALDWIN (1), A. E. Glenn (1)
(1) USDA, ARS, Toxicology & Mycotoxin Research Unit, Athens, GA, U.S.A.
Phytopathology 100:S10

Fusarium verticillioides is a non-obligate pathogen causing a number of maize diseases. Apart from these diseases, *F. verticillioides* is also known to

asymptomatically infect most tissues of the plant. The production of the mycotoxin fumonisin B1 by *F. verticillioides* and other complexities of the interactions with maize may contribute to the dual nature of this symbiont. One possible determinate of pathogenesis in the *F. verticillioides* – maize interaction could be the regulation and signaling by Reactive Nitrogen Species (RNS), specifically nitric oxide (NO). Detoxification of NO has been shown to be a pathogenicity factor for the fungal human pathogen *Candida albicans* and the bacterial plant pathogen *Erwinia chrysanthemi*. Both possess a flavohaemoglobin, encoded by *CaYHB1* and *HmpX*, respectively, that was determined to be responsible for this detoxification. BLASTP search of the *Fusarium* comparative genomes (Broad Institute) using these two genes revealed two putative homologs in *F. verticillioides*, denoted *NOD1* and *NOD2* (for Nitric Oxide Dioxygenase). To determine the function of *NOD1* and *NOD2*, each gene was individually deleted in *F. verticillioides* using PEG mediated transformation and homologous recombination. Mutants will be evaluated for their ability to detoxify NO and for virulence against maize seedlings. Understanding the function of these genes will give insight into the role of NO in the *F. verticillioides* – maize interaction.

Use of lesioned mutants to characterize the genetic network underlying control of the maize hypersensitive response

P. BALINT-KURTI (1), C. Weil (2), S. Chintamanani (3), R. Dhawan (4), A. Negeri (4), A. Garg (3), B. Venkata (3), J. Green (5), J. Harnsomburana (5), J. Palmer (2), V. Chaikam (2), C. Shyu (5), G. Johal (3)
(1) USDA-ARS, North Carolina State University, Raleigh, NC, U.S.A.; (2) Department of Agronomy, Purdue University, West Lafayette, IN, U.S.A.; (3) Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, U.S.A.; (4) Dept. of Plant Pathology, NCSU, Raleigh, NC, U.S.A.; (5) Informatics Institute, University of Missouri-Columbia, Columbia, MO, U.S.A.
Phytopathology 100:S11

The hypersensitive response (HR) is the most important defense response in plants, but details of how it is controlled and executed remain patchy. We used a novel genetic technique called MAGIC (Mutant-Assisted Gene Identification and Characterization) to identify an HR-modulating locus in maize. MAGIC facilitates identification of naturally-occurring alleles underlying phenotypic variation from diverse germplasm using a mutant phenotype as a "reporter". In this case the reporter phenotype is caused by a partially-dominant autoactive disease resistance gene, Rp1-D21, which causes HR lesions to form spontaneously. Genetic background profoundly affected the Rp1-D21 phenotype. B73 and Mo17 partially suppressed and enhanced the Rp1-D21 phenotype, respectively. By crossing the Rp1-D21 gene into a maize recombinant inbred line (RIL) mapping population, we were able to map and identify Hrml1 (HR-modulating locus 1), a locus responsible for modulating the Rp1-D21 phenotype, on chromosome 10. Loci with smaller effects were identified on chromosomes 1 and 9 (Genetics, in press). We are now extending these studies with much larger, mapping populations to uncover additional Hrml loci and to clone the underlying genes (funded by NSF grant #0822495). Furthermore, we are using computational image analysis to characterize the phenotypic expression of Rp1-D21 in diverse germplasm in different environments. We expect these studies to lead to a deeper understanding of the genetic network controlling the HR response in plants.

A protein localization and interaction map for Potato yellow dwarf virus, a plant-adapted nucleorhabdovirus

A. BANDYOPADHYAY (1), K. Kopperud (1), G. Anderson (1), K. Martin (1), M. Goodin (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S11

The complete genome of the type species of the genus Nucleorhabdovirus, Potato yellow dwarf virus (PYDV), was sequenced and functional protein assays were used to determine the subcellular localization of the proteins encoded by the virus. The antigenome of PYDV consists of 12,875 nucleotides and encodes 7 open reading frames (ORFs) equivalent to N (nucleocapsid), X (unknown), P (phosphoprotein), Y (unknown), M (matrix protein), G (glycoprotein) and L (polymerase) genes. The ORFs are separated by conserved intergenic junctions and are flanked by leader and trailer sequences, which are 149 and 91 nucleotides in length, respectively. When expressed in plant cells, the PYDV N, P and M proteins localized to nuclei, yet these proteins do not contain any predictable nuclear localization signals. In addition, the M protein was shown to induce the intranuclear accumulation of the inner nuclear membrane when expressed in the absence of any other viral protein. Bimolecular fluorescence complementation was used to generate the most comprehensive protein interaction map for a plant-adapted rhabdovirus to date. Furthermore, phylogenic analyses of L proteins indicated that PYDV is most closely related to the leafhopper-transmitted rhabdoviruses, which formed a clade distinct from those transmitted by aphids or planthoppers.

Mapping of genes for brown spot (*Bipolaris oryzae*) disease resistance in rice

S. P. BANU (1), B. Meah (2), D. S. Brar (3), H. Leung (3), C. M. VeraCruz (3)
(1) Bangladesh Agricultural Research Institute, Gazipur, BANGLADESH; (2) Bangladesh Agricultural University, Mymensingh, BANGLADESH; (3) International Rice Research Institute, PHILIPPINES
Phytopathology 100:S11

Brown spot (*Bipolaris oryzae*) is one of the major fungal diseases of rice and distributed world wide. A combination of genetic, molecular and pathological approaches was used in this study to identify and map novel, brown spot resistant genes. Traditional japonica cultivar Dinorado (resistant) crossed with semi dwarf modern indica cultivar IR 36 (susceptible). Phenotypic segregation of 200 F_3 progenies suggested that resistance to brown spot of rice is governed by two recessive genes with 1 (homozygous resistant) : 8 (heterozygous) : 7 (homozygous susceptible) ratio. In addition, corresponding F_2 progenies were used as mapping populations to identify DNA markers associated with resistance. Bulked segregant analysis was applied to analyze 186 F2 lines with 160 SSR markers distributed equally over each of the 12 chromosomes of rice genome. Marker analysis showed significant association (<.0001) for 4 markers and explained 16.53–48.17% of the total phenotypic variation for brown spot resistance. The interval analysis suggested that genes for resistance to brown spot are located between 8.7 and 18.2 MB in chromosome 12. The two genes imparting resistance to brown spot in Dinorado are designated as *bs1* and *bs2*. The present findings would provide guidelines to incorporate resistance from Dinorado to susceptible but otherwise high yielding cultivars of rice. The closely linked DNA markers would be suitable for use in marker-assisted selection in rice breeding programs.

Influence of temperature in the aquisition of '*Candidatus* Liberibacter americanus' by *Diaphorina citri*

J. C. BARBOSA (1), B. Eckstein (1), M. Gasparoto (1), R. da Silva (2), J. Belasque Jr. (2), A. Bergamin Filho (1)
(1) Esalq-USP, Piracicaba, BRAZIL; (2) Fundecitrus, Araraquara, BRAZIL
Phytopathology 100:S11

Huanglongbing is one of the most destructive diseases in citris in the world. Two species were detected in affected trees in Brazil: '*Candidatus* Liberibacter asiaticus' and '*Ca*. L. americanus' (CAM). Both species are transmitted by the psyllid *Diaphorina citri*. Studies carried out in Brazil suggest that CAM is sensitive to higher temperatures, and this characteristic have been associated to the reduction of the CAM infected plants incidence in São Paulo state, Brazil. However, the influence of temperature in the transmission of CAM by the psyllid is unknown. The objective of this work was to verify the influence of temperature in the acquisition of CAM by psyllids. In growth chamber, the acquisition of CAM by psyllids was analysed under three conditions of temperatures (20/22°C, 25/27°C and 30/32°C) in a 12 h photoperiod. For each temperature condition, groups of adult psyllids were caged on CAM infected citrus plants for an acquisition access period of four days. Psyllids were then transferred to citrus test-plants (*Citrus limonia* Osbeck). In each test-plant, one insect was confined for an inoculation access period of 24 days. After that, insects were collected and individually analyzed by PCR. Test-plants are kept in growth chamber at 25°C for future analyses. Under 20/25°C, the acquisition efficiency of CAM by psyllids was higher (49,21%) than at 25/27°C (40,97%) and 30/32°C (24,99%). Probably, these results may help explain the low incidence of CAM infected plants in Brazilian orchards nowadays.

Reliability and accuracy of visual methods used to quantify foliar symptoms of bacterial spot of peach and nectarine

S. J. Bardsley (1), H. K. NGUGI (1)
(1) Penn State University, Biglerville, PA, U.S.A.
Phytopathology 100:S11

Bacterial spot caused by *Xanthomonas arboricola* pv. *pruni* is the most important bacterial disease of peach and nectarine in Pennsylvania and severe epidemics can result in 100% yield loss. Studies on bacterial spot epidemiology rely on the quality of visual estimates of disease severity. The objective of this study was to assess the reliability and accuracy of visual estimates of bacterial spot severity compared to those of computer image analysis. Three sets of leaves (n = 103, 103, and 104 leaves) with disease severity levels ranging from 0 to 100% were assessed twice by one experienced rater using direct visual estimation of percent leaf area covered by symptoms. The leaves were also rated on a 1 to 7 rating scale (1 = 0% symptomatic area, and 7 = >45%) by the same rater. The same leaves were also assessed with the APS Assess image analysis software. Based on Lin's concordance analysis, direct estimation was more accurate than the use of the rating scale with Lin's concordance coefficient (ρ_c) values of 0.962, 0.957, and 0.945, respectively, for the three sets of leaf samples compared with 0.865, 0.921, and 0.816 for estimates based on the rating scale. Direct

estimation was more reliable, than the rating scale with the precision coefficient (*r*) values of 0.986, 0.990, and 0.948 compared with 0.962, 0.965, and 0.919 for the rating scale. Bacterial spot severity estimates based on direct estimation are more accurate and reliable than those based on the rating scale.

Effects of crop and environmental variables on sugarcane brown rust epidemics in Louisiana
W. BARRERA (1)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 100:S12

Brown rust, caused by *Puccinia melanocephala*, is one of the most important diseases of sugarcane worldwide. In Louisiana, yield losses exceeding 20% have been documented. Resistance has been the major means of disease control. However, pathogen adaptability can adversely affect resistance durability. Replacing cultivars during periodic disease outbreaks can be difficult due to a multiple year crop cycle and limited seedcane availability. The use of fungicides to limit losses has shown promising results. However, their use requires adequate understanding of the conditions leading to disease development to maximize the economic benefit. The objective is to determine the combination of crop growth characteristics and environmental factors that result in severe brown rust epidemics. The goals are to develop a model that describes disease progress and a forecasting system that provides for timely fungicide applications. Variables monitored at two locations during 2009 included leaf wetness, relative humidity, rainfall, ambient temperature, temperature at the leaf surface, wind direction, wind speed, solar radiation, plant height, shoot population, number of leaves per shoot, and crop canopy cover. Disease was assessed weekly as area exhibiting disease symptoms on selected leaves and percentage of pustules with sporulation. Variables most highly correlated with disease severity were shoot population, leaf wetness, and temperature inside the canopy. Additional results will be presented and discussed.

Chilling injury in tomatoes exposed to low temperatures in the field
J. A. BARTZ (1), M. A. Ritenour (2), M. Elkahky (3)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) University of Florida/Indian River Research and Education Center, Fort Pierce, FL, U.S.A.; (3) Plant Pathology Dept./University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S12

Winter tomato production in 2010 was marked by prolonged periods of low temperatures in the field. Harvested fruit exhibited surface pitting, puffiness and cat-facing. Ripened fruit developed high decay incidences (>20%) of Rhizopus rot and black rot (Alternaria). More decay was observed among fruit stored at higher humidity (>95% versus 90%). The final color of such fruit was marred by severe white/gold speckling in the periderm, which has been identified as calcium crystals (likely calcium oxalate). At least one tomato shipment was reported to have been rejected at a receiving point due to severe speckling. White speckling was reported in green fruit and gold in red fruit. We observed that removal of the surface tissue layers over the crystals changed the perceived color from gold or yellow to white. Calcium oxalate crystals are pyramids or needle shaped raphides. The latter cause damage to adjacent parenchyma cells as the fruit are jostled during harvest and handling. The damage promotes postharvest decays. Speckled fruit were reported to have a reduced shelf-life. Previously, speckles in tomato fruit was associated with reduced transpiration, high humidity, and warmer temperatures in the field or greenhouse and fertilizer ratios that increased calcium uptake by the plant. This is the first report that speckles can accompany chilling or near chilling temperatures during fruit production.

Controlling of fire blight on popular apple cultivars with M9 rootstock
K. BASTAS (1)
(1) Selcuk University Faculty of Agriculture, Konya, TURKEY
Phytopathology 100:S12

The shoot blight phase of fire blight caused by Erwinia amylovora is highly destructive within the current and subsequent growing seasons. This study gives an assessment of the effect of different preparats, harpin protein (Hp), γ aminobutryric acid+L-glutamic acid (ABA), copper oxychloride, copper sulphate pentahydrate, phosphoroz acid, Ca-nitrate, foshetyl-Al, prohexa-dione-Ca (PC), Bacillus subtilis, B. subtilis+coralline (Bsc), potassium+ sulphur (KS) on shoot growth and fire blight disease on 10 years old apple trees, Fuji, Breauburn, Royal Gala, Golden Delicious grafted on M9 rootstock in 2008 and 2009 years. Whilst PC treatments early in the season considerably decreased the length of shoots, Hp, Bsc, ABA, KS treatments caused increasing of shoot growth. Disease incidence in apple trees treated with PC, Hp and inoculated was about 18%, markedly contrasting with 62% in the untreated plants. The use of resistance-inducing substances during the early phase of shoot growth may offer a means of managing the shoot blight of fire blight disease on apples.

Characterization of extracytoplasmic function sigma factors in plant pathogenesis by *Pseudomonas syringae* pv. *syringae* B728a
P. BASU THAKUR (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S12

Pseudomonas syringae pv. *syringae* B728a, an aggressive bacterial pathogen of bean, utilizes large surface populations and extracellular signaling to initiate a fundamental change from an epiphytic to a pathogenic lifestyle. Extracytoplasmic function (ECF) sigma (σ) factors serve as important regulatory factors in responding to various environmental signals. Bioinformatic analysis of the B728a genome has revealed ten ECF σ factors, five of which have high levels of sequence similarity to the FecI-type of ECF σ factors and play a known role in the regulation of various iron transport systems. Because iron is essential for the induction of major virulence factors in B728a, we hypothesize that these FecI-type σ factors may play a critical role in the bacterium's transition between lifestyles. Deletion mutants of two FecI-type σ factors in B728a have been created using homologous recombination based on the phage λ Red recombinase method, and phenotypic analysis is being performed. In this report, we characterize the function, regulatory network and signal transduction mechanisms of these proteins to help describe the adaptation of B728a to a pathogenic lifestyle.

A new potyvirus infecting cantaloupe (*Cucumis melo*) in the Imperial Valley of California
O. BATUMAN (1), E. T. Natwick (2), R. L. Gilbertson (1)
(1) University of California, Davis, CA, U.S.A.; (2) University of California Cooperative Extension Imperial County, Holtville, CA, U.S.A.
Phytopathology 100:S12

In 2008, mosaic, veinal banding and leaf distortion symptoms were observed in cantaloupe in the Imperial Valley of California. Potyvirus infection was confirmed with PCR and degenerate primer pairs that target the HC-Pro and CI genes. The virus was sap-transmitted to *Chenopodium quinoa* (Willd.), *C. amaranticolor* L. and *Cucurbita pepo* cv. Small Sugar, but not to *Nicotiana benthamiana*, *Datura stramonium*, tomato, pepper and common bean. Sequence analysis of the complete viral RNA genome (~9.6 kb) revealed a typical potyvirus genome organization. The highest complete nucleotide sequence identity, 78%, was with *Zucchini yellow mosaic virus* (ZYMV). The capsid protein and 3′-untranslated regions were 92% identical to those of ZYMV, whereas HC-Pro and CI genes were 79% identical. Based on these results, this is apparently a new melon-infecting potyvirus species, which is most closely related to ZYMV.

Current status of benzimidazole resistance of *Erysiphe necator* in Virginia
A. B. BAUDOIN (1)
(1) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 100:S12

Benomyl was used on grapes in the U.S.A. since the early 1970s, against Botrytis, black rot, and powdery mildew (E. necator). Benomyl resistance of grape powdery mildew was documented in New York and existed but was rare in California in 1993–95. Benomyl was withdrawn in 2001, and thiophanate methyl received a grape tolerance in 2002 for control of the same diseases, but probably has received little use on Virginia grapes. No documentation of the presence and extent of benzimidazole resistance in Virginia *E. necator* could be found, although it is considered likely to be present. Bioassays were conducted with thiophanate methyl (Topsin M 70WP) to determine if it might be useful for occasional use. Data were obtained for 55 isolates from 19 Virginia and 4 nearby locations. Fifty-one of 55 *E. necator* isolates grew well on leaf tissue treated with 50 mg/liter a.i. of thiophanate methyl, two did not grow, and two had an intermediate reaction. A number of isolates were also tested against a different formulation of thiophanate methyl (Cleary 3336 Plus) and against an old sample of benomyl (Benlate 50DF). Cleary performed similarly to Topsin M, but several isolates were more strongly inhibited by benomyl. There appeared to be at least two different levels of benomyl resistance: isolates inhibited by 250 mg/liter a.i. but not by 50 mg/liter, and isolates inhibited by neither. *E. necator* resistance to thiophanate methyl appears to be widespread in Virginia.

Succinate Dehydrogenase Inhibitor resistance risk assessment studies on *Mycosphaerella graminicola* the causal agent of Septoria Leaf Blotch
C. Bayon (1), M. W. Fraaije (2), H. J. Cools (1), S. L. Rogers (1), J. A. Lucas (1), B. A. FRAAIJE (1)
(1) Rothamsted Research, Harpenden, UNITED KINGDOM; (2) University of Groningen, Groningen, NETHERLANDS
Phytopathology 100:S12

Mycosphaerella graminicola, causal agent of Septoria Leaf Blotch (SLB), is highly adaptable and has shown an ability to overcome host resistance and develop fungicide resistance. UK survey data from 1998 and 2004 has shown

that losses can exceed £40 million per year despite application of fungicides. Due to resistance development to MBC and QoI fungicides and reduced efficacy of some DMIs, SLB control is heavily dependent on robust rates of curative triazoles with chlorothalonil (a multi-site inhibitor) or boscalid (a Succinate Dehydrogenase Inhibitor (SDHI)) often added as mixing partner to ensure a high level of disease control and to reduce resistance risk. Several new SDHIs with some curative properties are expected to enter the market. Our aim is to develop tools to predict and monitor resistance development to SDHI fungicides in a range of cereal pathogens. We have generated and characterised a collection of carboxin-resistant UV-mutants of *M. graminicola*. A range of mutations (>13) located in Sdh subunits B, C and D have been identified and genotype-to-phenotype relationships established. Protein modelling and inhibitor docking studies were conducted to establish if SDHI inhibitors have different binding properties. Further diagnostic development, enabling detection of resistant alleles at low frequencies, and cross-resistance studies will aid implementation of anti-resistance strategies to prolong the cost-effectiveness and lifetime of SDHI fungicides.

Evaluation of seed treatments for management of *Rhizoctonia* damping-off in lettuce

F. BAYSAL-GUREL (1), S. A. Miller (1)
(1) The Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S13

Rhizoctonia damping-off can result in significant losses in lettuce transplant and direct seeded field production. Experiments were performed in a greenhouse to determine the efficacy of seed treatments against *Rhizoctonia* damping-off in lettuce. Treatments were Coronet, Coronet+Thiram and FarMore. Inoculum of *R. solani* was prepared on chopped potato/soil medium and incorporated into potting mix at the rate of 0.5 g/100 ml mix. The numbers of emerged healthy and diseased seedlings were counted 8, 15 and 22 days after seeding. The experiment was repeated twice. Emergence was significantly increased compared to the non-treated, inoculated control by all seed treatments in both experiments. The Coronet treatment alone and combined with Thiram increased the percentage of emerged and healthy seedlings to that observed in the non-inoculated control in the first experiment. These treatments were also significantly more effective than FarMore in increasing emergence. However, there were no significant differences between the treatments in the second experiment. Pre-emergence damping-off was moderate (24.5% and 14.6% in the non-treated, inoculated control in the first and second experiments, respectively). All of the treatments significantly reduced pre-emergence damping-off compared to the inoculated control. The fresh weight of lettuce seedlings was also significantly increased compared to the non-treated, inoculated control by all treatments.

Effect of disinfectants on transmission of *Clavibacter michiganensis* subsp. *michiganensis* during grafting

F. BAYSAL-GUREL (1), X. Xu (1), G. Rajashekara (1), S. A. Miller (1)
(1) The Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S13

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a serious disease of tomato produced in open field and protected environments worldwide. Typical symptoms are stunting, wilting and death of plants, foliar and stem necrosis and unmarketable fruit. Cmm is transmitted from infected seed to seedlings, and mechanically from plant to plant during grafting. Commercial disinfectants were examined for efficacy in eliminating Cmm from grafting tools that dispensed the disinfectants onto the cutting surface during grafting. A bioluminescent Cmm strain (BL-Cmm 17) was used to facilitate the study of Cmm movement through grafted plants. The movement of BL-Cmm17 up and down the stem from the graft union was determined by tissue imprinting the cut surface of both scion and rootstock on semi-selective medium at specific distances from the graft 0 and 7 days after grafting. KleenGrow, bleach and Virkon S disinfectants were effective in preventing Cmm transmission to grafted tomato seedlings without obvious phytotoxicity. These disinfectants may prevent grafting-mediated Cmm transmission when used in a grafting tool that delivers the disinfectant directly to the cutting surface.

Copper resistance in *Xanthomonas citri* subsp. *citri* (Xcc) and *X. alfalfae* subsp. *citrumelonis* (Xac) and comparison with other xanthomonads

F. BEHLAU (1), B. I. Canteros (2), J. H. Graham (3), J. B. Jones (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) INTA-EEA, Bella Vista, ARGENTINA; (3) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S13

Copper resistance genes (Cu^R) genes from Xcc strain A44 and Xac strain 1381 were cloned, sequenced and compared with xanthomonads from different locations. The genes *copL*, *copA*, *copB*, *copM*, *copG*, *copC* and *copD* were identified in Xcc A44. The same *cop* genes except *copC* and *copD* occurred in Xac 1381. In addition, *copF* was found downstream of *copG* in Xac 1381. *X. vesicatoria* 1111 and *Stenotrophomonas maltophilia* k279a had the same set of *cop* genes found in Xcc A44 and *copF*, located downstream of *copD*. A44 clone ends at *copD* and the cop genes in 1111 and k279a share high homology (>95%) with cop genes found in A44. Thus, we presume that *copF* is also present in A44. Primers based on the A44 gene sequences were used to PCR amplify *copL*, *copA* and *copB* from other Cu^R xanthomonads. Although sequence alignment of PCR products revealed high homology (>90%) for *copL*, *copA* and *copB* among different xanthomonads, phylogenetic analysis indicated variation. *Cop* genes in Xac strains from Florida were more diverse than in Xcc strains from Argentina. *Cop* genes in one Xcc strain were closely related to *X. vesicatoria* BV5-4 from Argentina while the cop genes in the remaining strains were closely related to *X. gardneri* from Costa Rica. Xac strains were not highly similar to one another and were grouped with other *Xanthomonas* spp. Cu^R genes in xanthomonads may have a common origin and have been exchanged by horizontal transfer.

Molecular analysis of turfgrass rusts reveals the widespread distribution of *Puccinia coronata* as a pathogen of Kentucky bluegrass

L. A. BEIRN (1), B. B. Clarke (1), J. Crouch (1)
(1) Rutgers University, New Brunswick, NJ, U.S.A.
Phytopathology 100:S13

Rust is a common disease of cultivated turfgrasses that can cause extensive damage in heavily infested areas. Over the past ten years, increased susceptibility has been observed among several Kentucky bluegrass (*Poa pratensis* L.) cultivars. To test whether increased disease in previously resistant cultivars could be the result of new rust species and/or shifts in rust race composition, wide-scale sampling of symptomatic grasses was conducted to identify the primary rust species associated with turfgrass hosts. Phylogenetic analysis of rDNA ITS sequences identified *Puccinia coronata*, *P. graminis*, and *P. striiformis* from the tissue sampled. *P. coronata* was the most prevalent species (68% of the samples) followed by *P. graminis* (27%) and *P. striiformis* (5%). These species frequencies contradict what has typically been reported by turfgrass breeders in the field based on phenotype and disease symptoms. Not only was *P. coronata* found to be the predominate species in the samples, but was also routinely found in association with Kentucky bluegrass, indicating that the most common traits used to identify these pathogens in the field - uredium/spore pigmentation and host plant association - are inadequate to accurately identify rust species. We used the ITS dataset to develop a real time PCR protocol as a tool for the accurate discrimination of these rusts from turf. AFLP analysis is currently in progress to evaluate genetic diversity in turfgrass rust populations.

Evaluation of synergistic effect between rhizobacterial strains *Fusarium oxysporum* biological control agents

J. C. Bejarano (1), S. Franco (1), P. JIMENEZ (1)
(1) Univ Militar Nueva Granada, Bogota, COLOMBIA
Phytopathology 100:S13

Using a bacteria consortium for biological control is a strategy that allows to explore posible synergistic relationships between the members of the consortium. Also, it has been debated that consortia stimulate the ecosystem diversity. Besides that, our model pathosystem (*Physalis peruviana*- *Fusarium oxysporum*) is economicaly important and with no pesticide residue tolerance in fruit. From a previous work, a collection of 5 rhizobacteria (2 *Bacillus subtilis*, 2 *Pseudomonas fluorescens* and 1 *Pseudomonas* sp.) was obtained. These 5 bacteria were distributed in consortia of 1, 2, 3, 4, 5 bacteria, and their effect on *F. oxysporum* radial growth, the number of macro and micro conidia produced per colonial area, and on *P. peruviana* germination rate, were evaluated. Results showed that different bacterial combinations present synergistic effect for an activity but they did not work well facing other challenges. Concerning the results of the combinations, we found that increasing the number of members in the consortium could have an inhibitory effect among the bacteria especies. This effect is probably due to the fact that metabolite production, such as antibiotics, is stimulated by processes such as competence for nutritional resources, resulting then in an antagonistic effect.

PCR-RFLP analysis on genetic diversity of *Fusarium* spp. isolates collected from sugarbeet fields of Iran

S. Beladi (1), S. REZAEE (1), B. Mahmoodi (2)
(1) Dept. of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Sugar Beet Seed Institute, Karaj, IRAN
Phytopathology 100:S13

The amplified ITS region of rDNA, digested with three restriction endonucleases; EcoR1, Taq1 and HaeIII, to analyze the genetic diversity among 28 *Fusarium* spp. isolates from diseased sugarbeet with wilting and yellowing symptoms from nine different regions of Iran. Based on morphological features, 13 *Fusarium oxysporum*, seven *Fusarium solani* and eight *Fusarium proliferatum* isolates were identified. Using PCR method two

fragments of 550 and 570 bp were amplified for ITS1 and ITS4 sequences of all isolates. Digestion patterns of ITS amplification products with EcoR1 revealed one restriction site in this region of DNA. No clear rDNA polymorphism was observed after digestion with EcoR1. Digestion with Tag1 and HaeIII showed genetic diversity among different species of *Fusarium*. Digestion with HaeIII resulted in three banding patterns with two or three restriction sites. Digestion with Taq1 exhibited three banding patterns with three or four bands with different sizes. The cluster analysis separated all isolates into three groups based on their species. According to these results, Iranian *Fusarium* isolates can be separated easily into different species on the basis of ITS-RFLP method.

Sensitivity of *Alternaria solani* populations in Idaho to commonly used fungicides and its effect on potato early blight management in Idaho

A. R. BELCHER (1), E. Wood (1), P. S. Wharton (1)
(1) University of Idaho, Aberdeen, ID, U.S.A.
Phytopathology 100:S14

Early blight, caused by the fungus *Alternaria solani*, is an important disease on potato in Idaho. The strobilurin (Q_oI) fungicides (e.g. azoxystrobin and pyraclostrobin) are currently favored as effective and safe methods for the control of early blight. Q_oI fungicides were registered for use on potato in the U.S. in the late 1990s. Soon after, in the early 2000s, isolates of *A. solani* with reduced sensitivity to the Q_oIs were detected across the Midwest. In 2007 and 2008 many Idaho potato growers reported the failure of these fungicides to control early blight. Thus, the goal of this project was to assess the prevalence of *A. solani* isolates with reduced sensitivity to the Q_oI fungicides in southeastern Idaho. In 2009, 77 isolates were collected from leaves and tubers of potatoes grown in experimental and commercial fields. The isolates were tested for sensitivity to azoxystrobin, pyraclostrobin, famoxodone plus cymoxanil, and boscalid. Fungicide solutions were applied to PDA in a 2.5-log dilution in a continuous radial concentration gradient using a spiral plater. Fungal inoculum was placed in radial lines across the gradient. Fungicide sensitivity was expressed as an EC_{50}, the fungicide concentration at which a fungal isolate's radial growth was equal to 50% of the average growth of the isolate on non-amended PDA. The results, to be presented, will be used to develop a more sustainable potato early blight control program for Idaho growers.

The effect of foliar fungicides on yield across Iowa in the 2008 and 2009 growing seasons

N. R. BESTOR (1), D. S. Mueller (1), A. E. Robertson (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S14

Interest in the use of foliar fungicides of soybean has increased in the past five years. Reasons for this include higher soybean prices and the threat of foliar diseases. In Iowa, diseases such as brown spot (Septoria glycines), Cercospora leaf blight (Cercospora kikuchii) and frogeye leaf spot (C. sojina) can potentially reduce yields. The effect of a foliar fungicide applied at either growth stage R1 or R3 on disease severity and yield of soybean was evaluated at five locations in Iowa in both 2008 and 2009. Fungicides belonging to strobilurin, triazole and carboxamide groups were evaluated. Percent foliar disease of brown spot, Cercospora leaf blight, frogeye leaf spot, and downy mildew were assessed at growth stage R6. Brown spot was the most prevalent disease at all locations, but disease pressure was low in both 2008 and 2009 (15% severity and 5.5% severity in the lower canopy at growth stage R6, respectively). In general, yields were greatest with an R3 application of a fungicide containing a strobilurin. In 2008, the average of all fungicides at all five locations applied at R3 (54.3 bu/ac) yielded higher than the non-treated control (50.2 bu/ac). However, in 2009, the average of all fungicides at all five locations applied at R3 (60.7 bu/ac) yielded similar to the non-treated control (60.1 bu/ac).

Identification and characterization of fungal communities associated with soybean roots in Minnesota

J. C. BIENAPFL (1), D. K. Malvick (1), J. A. Percich (1)
(1) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 100:S14

Root diseases of soybean cause substantial yield reduction in the U.S. However, information regarding the distribution and pathogenicity of fungal communities associated with soybean roots is limited. In 2007 and 2008, soybean root samples were collected in July from 15 fields in 10 counties representing major soybean production areas in Minnesota (MN). Symptomatic and asymptomatic root tissue was plated onto four different media. Although *Fusarium* was the most frequently isolated genus in all locations, additional fungal genera and species were recovered. Thirty representative non-*Fusarium* isolates were selected and identified to species using morphological characteristics and sequences of the internal transcribed spacer region. Pathogenicity of these isolates to soybean seedlings was evaluated in a greenhouse. The 30 fungal isolates included the genera *Arthrographis*, *Bionectria*, *Cylindrocarpon*, *Exophiala*, *Neonectria*, *Mortierella*, and *Rhizoctonia*. Results indicated a diversity of fungi is associated with soybean roots in the early reproductive stages in MN, including some that have not been previously reported from soybean roots. Isolates of *Arthrographis*, *Bionectria*, *Cylindrocarpon*, *Exophiala*, *Neonectria*, and *Rhizoctonia* species produced root rot symptoms ranging from discrete lesions to extensive taproot necrosis on seedlings and may cause root rot in MN soybean fields. Additional isolates are currently being identified and tested for pathogenicity on soybean.

Development of a multiplex assay for genus and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order

G. BILODEAU (1), F. N. Martin (1), M. D. Coffey (2), C. L. Blomquist (3)
(1) United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Salinas, CA, U.S.A.; (2) Department of Plant Pathology and Microbiology, University of California Riverside, Riverside, CA, U.S.A.; (3) California Department of Food and Agriculture, Plant Pest Diagnostics Branch, Sacramento, CA, U.S.A.
Phytopathology 100:S14

The genus *Phytophthora* contains more than one hundred described species and given their importance to agriculture accurate and rapid detection tools are essential. A range of markers have been developed for this genus, usually based on polymorphisms at primer annealing sites that rely on accurate control of annealing temperature for specificity. An alternative approach for enhanced specificity is to design markers based on differences in the location of annealing sites. We have looked at gene order differences in the mitochondrial genome of *Phytophthora* compared to *Pythium* and plants for developing a single amplification assay for genus as well as species specific detection (single amplification primer pair with TaqMan probes for genus and species-specific ID). Three conserved gene order differences have been identified with conserved regions suitable for genus specific detection adjacent to variable regions for species-specificity. Two of these should allow for design of species-specific probes for more than 65 species. The amplification primers and genus specific probe were effective when evaluated against a wide range of isolates representing all formally and provisionally described *Phytophthora* spp. as well as a number of *Pythium* spp. and plants. Multiplex amplifications with species-specific probe combinations *P. ramorum-kernoviae*, *P. fragariae-citricola-cactorum* and *P. alni* were also effective and are under evaluation with field samples.

Quantification and rapid detection of *Verticillium dahliae* in soil

G. BILODEAU (1), P. Uribe (1), S. T. Koike (2), F. Martin (1)
(1) United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Salinas, CA, U.S.A.; (2) Univ. Calif. Cooperative Extension, Monterey County, Salinas, CA, U.S.A.
Phytopathology 100:S14

Verticillium wilt is caused by the fungus *Verticillium dahliae*, which survives in the soil for long periods of time as microsclerotia. In strawberry production inoculum densities of 3-5 ms/g are high enough to cause disease losses. A soil-plating assay is currently used to assess inoculum density but takes 6–8 weeks to get results. The development of a rapid and accurate detection method using a molecular diagnostic assay like TaqMan Real-Time PCR is desirable. We have approached this objective by focusing on optimizing procedures for DNA extraction from the soil, developing post extraction procedures to remove PCR inhibitors, and creating a highly sensitive marker system specific for *V. dahliae*. Soil quantification with molecular techniques is challenging because PCR inhibitors can influence amplification kinetics, making accurate quantification across samples or soil types difficult. To account for this an internal control assay multiplexed with the *V. dahliae* assay was developed. To correlate the results of the PCR assay with soil population densities, soil samples were collected from infested fields and analyzed by traditional plate count technique and DNA extraction using the optimized real-time PCR assay. The results obtained with the two methods were plotted using regression analysis and the correlation between the Ct value and the plate count was high ($R^2 = 0.85$). Pathogen densities as low as 2 ms/g soil were accurately detected with a final Ct less than 34.

Effect of temperature on Bacterial wilt, caused by *Ralstonia solanacearum*, incidence in tobacco cultivars

R. J. BITTNER (1), A. Mila (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S14

Bacterial wilt is a destructive disease of many crops, including tobacco. Use of resistant cultivars is one of the most effective means to reduce losses from R. solanacearum, but little is known about the mechanism of resistance in tobacco cultivars. Temperatures above 28°C have been shown to increase the

severity of bacterial wilt in resistant tobacco cultivars. In this study, we examined the effect of different temperatures on resistance of six tobacco cultivars to R. solanacearum. Highly resistant cultivars K346 and Sp168 and low resistant cultivars K326, NC71, RJR15, and RJR75 were compared under six temperatures (35, 30, 25, 20, 15, and 10°C). Four strains of R. solanacearum (race 1, biovar 1) were used. The highest disease incidence was observed in all cultivars at 30 and 35°C, 18 days post-inoculation. In contrast, no disease symptoms were observed when plants were incubated at 10 and 15°C. Plants were placed in a 30°C incubator for an additional 18 days and disease was observed on all cultivars. Temperature ($P < 0.0001$), cultivar ($P = 0.03$), and strain ($P < 0.0001$) were significant factors explaining disease incidence. We are currently further investigating if the temperature affects the host (cultivar), the pathogen strain, or their interaction. Understanding host parasite interactions and temperature effects offers information to advance breeding and disease management strategies.

Diversity of sooty blotch and flyspeck fungi from apples in northeastern Turkey

J. M. BLASER (1), A. Karakaya (2), D. A. Mayfield (1), J. C. Batzer (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.; (2) University of Ankara, Ankara, TURKEY
Phytopathology 100:S15

Fungi in the sooty blotch and flyspeck (SBFS) complex are epiphytes that infest apples and other tree fruit crops. In regions with humid climates, SBFS fungi blemish the fruit cuticle, reducing market value of the crop. In this study, SBFS isolates were obtained from apples grown in Rize, Turkey. SBFS colonies with subtending apple cuticle were excised, pressed, photographed, and shipped to Ames, Iowa for isolation. Of 592 primary isolates from 148 apple peels, 50 isolates were selected for further study. The internal transcriber spacer (ITS) and large subunit (LSU) regions of ribosomal DNA were amplified, sequenced and compared to previously identified fungi. Isolates were placed into genera using parsimony analysis of the LSU. Putative species were delineated from ITS sequences as well as morphology on apple and in culture. A total of 17 putative species were delineated; 14 were placed in the Capnodiales, two were placed in the Chaetothyriales, and one could not be placed to order but LSU parsimony analysis grouped it in Dothideomycetes. Eleven putative SBFS species had not been described previously, whereas previously described species included *Peltaster fructicola*, *Zygophiala wisconsinensis*, *Pseudocercosporella* spp. RH1 and RH3.1, *Zygophiala* sp. FS6, and *Stomiopeltis* sp. RS4.1. These findings expand the documented range of genetic diversity within the SBFS complex and are the first information about the taxonomic classification of these fungi from Turkey.

The activity of citrus canker lesions on grapefruit in Florida, June 2009–January 2010

C. H. BOCK (1), P. E. Parker (2), T. R. Gottwald (3), J. H. Graham (4)
(1) USDA ARS, Fort Pierce, FL, U.S.A.; (2) USDA-APHIS-PPQ, Edinburg, TX, U.S.A.; (3) USDA, ARS, USHRL, Fort Pierce, FL, U.S.A.; (4) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S15

Lesions of citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), on citrus fruit preclude sale of the fruit to the fresh market. Assessing lesion activity in orchard-grown grapefruit provides information on the population dynamics of fruit lesions in a commercial situation and helps gauge risk associated with infected fruit entering fresh markets. To quantify the proportion of active lesions, and Xcc production, we collected eighty lesions from the rind of grapefruit once a month from June 2009–January 2010 from an orchard in East Florida and assessed activity of each lesion by dilution plating on nutrient agar. Linear regression analysis indicated a slight decline in the proportion of active lesions ($R2 = 0.45$). In June 88% of lesions produced Xcc, and by January, at the time of harvest, 69% of lesions were active. However, the average number of bacteria produced was greatest in November (3.9×105 Xcc/mm2 of lesion), and least in August (2.8×104 Xcc/mm2 of lesion). In January, 2.0×105 Xcc/mm2 lesion was produced. The maximum quantity of Xcc produced was in December (5.2×107 Xcc/mm2 of lesion). These data suggest that in Florida there was little change in the activity of canker lesions on fruit from shortly after lesion development to the point of harvest. Foliar lesions are also reported to remain fully active for at least six months. This reinforces the need to focus on post harvest approaches to deactivating lesions of citrus canker on fresh fruit.

Distribution of canker lesions on grapefruit in Florida

C. H. BOCK (1), P. E. Parker (2), T. R. Gottwald (3)
(1) USDA ARS, Ft Pierce, FL, U.S.A.; (2) USDA-APHIS-PPQ, Edinburg, TX, U.S.A.; (3) USDA, ARS, USHRL, Fort Pierce, FL, U.S.A.
Phytopathology 100:S15

Citrus canker, caused by the plant pathogenic bacterium Xanthomonas citri subsp. citri (Xcc) is an important disease of grapfruit in Florida. To establish disease distribution on fruit, six samples of 24 diseased grapefruit were collected from two groves in East Florida. A plane was sliced through the middle of the fruit such that the vertical dimension of the fruit (the diameter, with peduncle at the apex) was split into equal sections. The surface area of each fruit half (hemisphere) was assumed to be the same. For all six samples of 24 fruit each the lesions were enumerated on the upper (peduncle end) and lower (flower scar) halves. On four of the samples the fruit sliced along three planes and the number of lesions enumerated on each of the four slices. On the six samples, 70–82% of all lesions were found on the upper half of the grapefruit. Sequentially on the four quartered samples, 40–47% of all lesions were found on the upper quarter of the fruit, and 28–39%, 9–16% and 7–10% of lesions were found on the lower three quarters, respectively. GLM analysis showed significant differences in lesion counts from each section; the highest count consistently being on the upper portions of the fruit. A logistic model described the relationship between lesion count and vertical distance from the fruit apex. Presumably the upper surfaces of the fruit are more prone to infection as they have greater exposure to splash born inoculum.

Proteomics analysis of *Ralstonia solanacearum* identifies candidate proteins that contribute to pathogenicity on tomato plants at low temperatures

A. BOCSANCZY (1), U. C. Achenbach (2), D. J. Norman (1)
(1) Department of Plant Pathology, University of Florida, IFAS-MREC, Apopka, FL, U.S.A.; (2) Institute of Molecular Plant Physiology and Biotechnology of Plants (IMBIO), Bonn, GERMANY
Phytopathology 100:S15

Ralstonia solanacearum is a common bacterial plant pathogen in tropical and subtropical areas of the world with a wide host range which include economically important crops such as potato and tomato. The pathogen species is very diverse and complex. *R. solanacearum* strain UW551 which has been classified as a race 3 biovar 2 (R3B2) has the ability to infect tomato and potato plants at low temperatures as we previously determined comparing the pathogenicity of R3B2 strains with other races of *Ralstonia* in environmental chambers at 18°C and at 30°C. The model strain GMI1000 however does not infect its host plants at 18°C. We hypothesized that either UW551 has genes not present in GMI1000 or their expression is differentially regulated at low temperatures. The objective of our study is to identify determinants at molecular level of the pathogenicity of UW551 at low temperatures. In order to identify candidates for genes/ proteins we designed experiments where we compared protein levels of *R. solanacearum* UW551 and GMI1000 at 18°C and 30°C, when the pathogenic strains are in contact with tomato plants roots. We identified a list of potential candidate genes that might contribute to the virulence of UW551 under cool weather conditions. Currently we are confirming expression of the candidate genes using real time PCR techniques and we are working towards producing deletion mutants of selected candidates in order to characterize their function in pathogenicity.

Field detection of *Phytophthora ramorum* DNA within 30 minutes

R. C. BOHANNON (1), P. Russell (1)
(1) Agdia Inc., Elkhart, IN, U.S.A.
Phytopathology 100:S15

Agdia, Inc. has developed a method to detect plant pathogen DNA in less than 30 minutes, start to finish, using a new isothermal DNA amplification system. Initial tests were developed to detect *Phythophthora ramorum* as the prototypic target analyte, owing to its importance in the ornamental field and association with Sudden Oak Death syndrome…responsible for killing millions of trees in California and Oregon. The new system successfully demonstrated 100% specificity for *P. ramorum* in over 70 samples tested from *Pythophtora and Pythium* lineages and demonstrated extremely low levels of detection. Tests were developed against other relevant pathogens and a system developed to give users an answer in the field, or lab, in less than 5 minutes without the need for instrumentation for selected analytes. Discussion of this breakthrough and technology will be presented.

Modeling Fusarium head blight and deoxynivalenol content in barley in response to field temperature and wetness durations

K. D. BONDALAPATI (1), J. M. Stein (1)
(1) South Dakota State University, Brookings, SD, U.S.A.
Phytopathology 100:S15

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae* (anamorph: *Fusarium graminearum*), continues to be a serious problem for barley producers in the U.S. Northern Great Plains and elsewhere. Field experiments were conducted during the 2005–9 growing seasons to evaluate the combined effects of temperature and wetness durations (relative humidity > 90%) prior to full head emergence on disease development and deoxynivalenol (DON) accumulation in malting barley. Disease incidence

(number of diseased spikes/total), average severity (number of disease spikelets/total), and DON content in the grain (mg/kg) were collected from 51 location*years for three varieties, 'Conlon', 'Robust' and 'Tradition'. A binary DON response variable, eDON, was created based on the DON content for each variety at every location*year where '0' and '1' represent values below or above a threshold of 0.5 mg/kg. A Weibull function was calculated to predict the probability of infection using the average temperature and weighted wetness durations in the field during the 10-day prior and including the full head emergence day. Disease incidence, severity, DON content and eDON were significantly correlated to the Weibull variable calculated from the field weather data ($p < 0.001$). The sensitivity, specificity and total prediction accuracy obtained from the confusion matrix after dividing the observations at a cut-off of 0.5 of Weibull variable in comparison with eDON were greater than 80%.

Effects of water vapor, liquid water, and their interaction on the germination of urediniospores of *Phakopsora pachyrhizi*
M. R. BONDE (1)
(1) USDA ARS, Frederick, MD, U.S.A.
Phytopathology 100:S16

"Cold-induced dormancy" has been described as a phenomenon in which urediniospores, including those of *P. pachyrhizi*, after having been frozen at ultra-low temperatures, require either a heat shock (e.g. 40°C for 5 min) or over night hydration in a water-saturated atmosphere to germinate. To better understand this phenomenon in *P. pachyrhizi*, following liquid nitrogen storage urediniospores were subjected to specific treatments including over night hydration in a water-saturated atmosphere, a 40°C-heat shock for 5 min, and submersion in liquid water (0.02% Tween 20), individually and in every treatment combination. Fresh urediniospores from plants in a containment greenhouse served as controls. In all experiments, fresh and previously frozen urediniospores behaved the same. Heat shocks had no effect on germination, whereas hydration in a water-saturated atmosphere was required for high germination for all samples. Submersion of urediniospores in liquid water without prior hydration in a water-saturated atmosphere reduced germination by 84 to 96%, whereas floating non-hydrated urediniospores on liquid water reduced germination by only 45% compared to hydrated urediniospores placed directly on water agar. When hydrated urediniospores were submerged for 2 min to 60 min in liquid water, germination was reduced only by 11 to 23%, respectively. The interaction of water vapor and liquid water in the laboratory was highly significant and may play an important role in nature.

An assessment of sensitivity to fungicides in Tennessee isolates of the cucurbit powdery mildew pathogen, *Podosphaera xanthii*
S. C. BOST (1)
(1) University of Tennessee, Nashville, TN, U.S.A.
Phytopathology 100:S16

Seven Tennessee isolates of the cucurbit powdery mildew pathogen, *Podosphaera xanthii*, were tested for sensitivity to several fungicides in 2009. Comparison of the results to two earlier surveys (1998 and 2004) indicates that the incidence of high levels of resistance to the strobilurins increased from zero in 1998 to 100 percent in 2009. Systemic fungicides had not been used in any of the fields sampled in 2009, and two of the fields were organic production. High levels of resistance to the benzimidazoles were found in all isolates in all three surveys. All isolates tested in the 2009 survey were highly sensitive to myclobutanil, boscalid, sulfur, cyprodinil plus fludioxonil, fenthiopyrid, and quinoxyfen. However, the survey results conflicted with recent anecdotal field reports indicating undesirable levels of control by myclobutanil. The powdery mildew population present in a fungicide efficacy field trial did not change in sensitivity to any of the tested products, based on the results of greenhouse tests conducted on samples collected before the first application and after the final application.

Sorghum as a bioenergy crop in Alabama: Disease and yield evaluations
K. L. BOWEN (1), A. K. Hagan (1), A. C. Rocateli (2), R. L. Raper (3), E. B. Schwab (4), D. Bransby (2), F. J. Arriaga (5), K. S. Balkcom (5)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Agronomy and Soils, Auburn University, AL, U.S.A.; (3) USDA-ARS Dale Bumpers Research Center, Booneville, AR, U.S.A.; (4) USDA-NRCS, Auburn, AL, U.S.A.; (5) USDA-ARS National Soil Dynamics Lab, Auburn, AL, U.S.A.
Phytopathology 100:S16

In Alabama, in 2009, several studies were monitored to evaluate the impact of various production practices on disease occurrence and the effect of diseases on yields of sorghum. Sweet sorghum cultivars, as well as grain and forage sorghum, were included in these studies. Fresh (biomass) and dry weights as well as juice and Brix (% sucrose) yields were recorded. In south-central Alabama (Brewton), the best cultivars, including 'M81-E' and 'Dale', yielded in excess of 80 tons per hectare fresh weight biomass in the absence of diseases. Zonate leaf spot (*Gloeocercospora sorghi*) was the dominant disease in south Alabama (Baldwin Co.); however, anthracnose (*Colletotrichum graminicola*) occurred primarily on the sweet sorghum M81-E. Anthracnose also predominated on sorghum in east-central AL (Macon Co.). In one Macon Co. trial, anthracnose was lower in conventionally tilled plots than those under conservation tillage. Brix was negatively related to anthracnose intensity on M81-E in south AL, while this dry matter yield was negatively related to anthracnose in east-central AL on forage sorghum. Increasing nitrogen rates had little if any impact on disease severity or any yield parameter in south AL. However, more severe anthracnose was found with higher nitrogen rates and with later planting dates in sweet sorghum in Macon Co.

Evaluating the impact of nutritional treatments on *Xylella fastidiosa* in grapevine
J. BRADY (1), J. Faske (1), T. Faske (2), D. McGahan (2)
(1) Texas AgriLife Research, Stephenville, TX, U.S.A.; (2) Tarleton State University, Stephenville, TX, U.S.A.
Phytopathology 100:S16

Xylella fastidiosa is a bacterial pathogen that causes leaf scorch in a wide array of plant species including grape, where it causes Pierce's disease. *X. fastidiosa* colonizes the nutritionally poor environment of xylem elements, utilizing dilute salts and amino acids in xylem fluid. The bacterium exhibits fastidious nutritional requirements, a slow growth rate, and is sensitive to perturbations in its growth medium. Plant nutritional regimes were examined for the potential to alter bacterial proliferation *in planta*. Potted Cabernet Sauvignon grapevines were mechanically inoculated with *X. fastidiosa* and subjected to weekly soil drench treatments in which levels of selected micronutrients were varied, either alone or as a component of Hoagland's solution #2. *X. fastidiosa* proliferation in stem tissue was measured by QRT-PCR. Grapevines treated with increased levels of zinc, manganese, or copper had fewer *X. fastidiosa* than grapevines treated with Hoagland's solution or water. Grapevines treated with Hoagland's solution minus zinc had higher *X. fastidiosa* levels than grapevines treated with complete Hoagland's solution, while bacterial levels in manganese deprived grapevines were not different than those treated with complete Hoagland's solution. Experiments are ongoing to test the utility of altered plant nutritional regimes as a management tactic in field plots.

Phytotoxicity danger of phosphorous acid generating fungicides and fertilizer products applied to blueberry and grapes
P. M. BRANNEN (1), J. Garner (2), J. Smith (3)
(1) University of Georgia, Athens, GA, U.S.A.; (2) University of Georgia, Blairsville, GA, U.S.A.; (3) University of Georgia, Alma, GA, U.S.A.
Phytopathology 100:S16

Phosphorous acid generating fungicides and fertilizers have seen a recent increase in use for either direct of indirect management of multiple diseases of small fruits, especially Phytophthora root rot and downy mildew. Labels differ markedly between phosphorous acid products, and though these products are known to have some phytotoxic issues, label warnings and use patterns vary between products; labeled spray intervals and rates are highly variable for the same commodities, though near-equivalent amounts of phosphorous acid might be utilized. In some cases, overuse of these materials by producers has been observed. To confirm potential phytotoxic responses with phosphorous acid applied to blueberry, studies in Georgia with weekly applications of phosphorous acid indicated that damage did not necessarily occur gradually, but damage, as indicated by scorched leaves and leaf drop, was observed rapidly following five applications of the material. Likewise, similar damage was observed in *Vitis vinifera* grapes in two years of testing, with 100% plant mortality occurring in the second year of testing after six applications. Similarly, scorch and plant mortality did not occur gradually, but was only observed after an apparent critical plant stress was achieved. Phosphorous acid generators are excellent tools for disease management, but their phytotoxic potential should be further tested, clearly communicated, and potentially standardized across products.

Aggressiveness of *Rhizoctonia solani* AG 2-2 on sugar beet and rotation crops
J. R. BRANTNER (1), C. E. Windels (1)
(1) University of Minnesota, NWROC, Crookston, MN, U.S.A.
Phytopathology 100:S16

Rhizoctonia crown and root rot of sugar beet caused by *Rhizoctonia solani* AG 2-2 intraspecific groups (ISGs) IV and IIIB is increasing in Minnesota and North Dakota. Of 1,000 cultures of AG 2-2 isolated from diseased sugar beet, 24 of each ISG were selected to represent a wide geographic area and previous crops. They were tested for aggressiveness on adult sugar beet roots and seedlings of sugar beet and rotation crops. Adult sugar beet roots were inoculated when 8-wk old; in seedling tests, a commercial greenhouse soil was infested with inoculum before planting. Disease was assessed at 12 days after inoculation. Both ISGs were equally aggressive on adult sugar beet roots;

root rot indices (RRI = 0-7 scale) averaged 5.0 (3.3-5.6) for IV and 4.9 (3.8-5.9) for IIIB. On seedlings of sugar beet and rotation crops, range of aggressiveness for both ISGs overlapped, but IIIB caused significantly more disease than IV. Sugar beet RRI (0-100 scale) averaged 78 (42-100) for IIIB and 51 (5-100) for IV. Corn RRI (1-5 scale) averaged 3.1 (1.8-4.1) for IIIB and 2.1 (1.2-3.0) for IV. Pinto bean RRI (1-5 scale) averaged 4.4 (3.5-5.0) for IIIB and 2.7 (1.8-4.5) for IV. Soybean RRI (1-5 scale) averaged 4.1 (2.7-5.0) for IIIB and 3.2 (1.9-4.6) for IV. Aggressiveness of AG 2-2 cultures on adult sugar beet roots was unrelated to pathogenicity on seedlings of any crop but there were significant correlations ($P < 0.001$) for aggressiveness of cultures on seedlings between crops.

The response of the energy crop Miscanthus to fungal pathogens: A preliminary study

J. M. BRENNAN (1), E. Glynn (2), K. McDonnell (3)
(1) Plant Health Laboratory, Seed Certification Division, Department of Agriculture, Fisheries and Food, Backweston, Celbridge, Kildare, IRELAND; (2) Department of Agriculture, Fisheries and Food, Backweston & UCD School of Agriculture, Food Science and Veterinary Medicine, UCD College of Life Sciences, Belfield, Dublin 4, Kildare, IRELAND; (3) UCD School of Agriculture, Food Science and Veterinary Medicine, UCD College of Life Sciences, Belfield, Dublin 4, Dublin, IRELAND
Phytopathology 100:S17

There is increasing interest in the use of biomass crops for energy production. Miscanthus is a perennial rhizomatous C4 grass that has remarkable adaptability to different climatic environments. As the acreage of the biofuel crop Miscanthus expands in Ireland and the EU, we can expect that pests and pathogens will have a significant impact on both biomass yield and biomass quality. However, little is known about diseases of novel biofuel crops such as Miscanthus. The aim of this research was to assess the response of Miscanthus x giganteus towards fungal pathogens including some of the main Irish cereal pathogens using an *in vitro* detached leaf assay and *in vivo* whole plant test. Visual disease symptoms observed on the Miscanthus x giganteus leaves in this experiment varied among the 19 pathogens (37 isolates) assessed and symptoms included brown lesions, premature necrosis, presence of pynidia and mycelial growth. In general, all 19 pathogens (37 isolates) assessed caused some level of disease *in vitro*. The pathogen *Rhizoctonia solani* caused the greatest visual disease symptoms on the Miscanthus x giganteus leaves (mean LGR = 3.5 cm day -1), while *R. secalis* caused the least visual disease symptoms (mean LGR = 1.5 cm day -1) ($P < 0.05$). While of the 6 pathogens assessed in the *in vivo* whole plant test only *F. culmorum*, *F. graminearum* and *M. nivale* caused visual disease symptoms (mean LGR = 0.3 – 0.5 cm day -1).

***Phytophthora ramorum* and *Phytophthora kernoviae* in Ireland: The current situation**

J. BRENNAN (1), D. Cummins (1), S. Kearney (1), G. Cahalane (1), S. Nolan (1), J. Choiseul (1)
(1) Department of Agriculture, Fisheries & Food, Kildare, IRELAND
Phytopathology 100:S17

Phytophthora ramorum is a serious pathogen of trees and ornamental plants, causing a disease known as sudden oak death (SOD) in the U.S.A. Plants affected by *P. ramorum* show a range of symptoms including stem canker and tip dieback. *Phytophthora ramorum* was first detected Ireland in 2003. *Phytophthora ramorum* is reported to infect over 64 plant species, including a number which have significant commercial and amenity value in Ireland, particularly Rhododendron and Viburnum spp. The closely-related *P. kernoviae* causes similar symptoms to *P. ramorum* and was first discovered in the UK in 2003, New Zealand in 2006 and Ireland in 2009. To date *P. ramorum* and *P. kernoviae* have not been detected on tree species in Ireland, however there is strong concern however that Irish trees could become infected. Extensive surveys have been carried out by the Department of Agriculture, Fisheries & Food (DAFF) from 2003 to present. Since 2003 nearly 6000 samples were collected around Ireland and *P. ramorum* was detected in all years: positive samples: 8% (2003), 2% (2004), 19% (2005), 10% (2006) & 16% (2007), 12% (2008) & 11% (2009)]. In 2003, *P. ramorum* was only found on Rhododendron and Viburnum spp., by 2009 the presence of *P. ramorum* was confirmed on six plant genera (Rhododendron, Viburnum, Camellia, Photinia, Magnolia & Leucothoe). *Phytophthora kernoviae* was first detected in Ireland in 2008 on Rhododendron spp. and confirmed in 2009. Eradication & containment measures are being implemented in accordance with EU legislation.

Discovering single nucleotide polymorphisms (SNPs) in an uncharacterized fungal genome using the software EagleView to evaluate 454 sequencing data

K. D. BRODERS (1), P. J. SanMiguel (2), R. P. Westerman (2), K. E. Woeste (3), G. J. Boland (1)
(1) School of Environmental Sciences, University of Guelph, Guelph, ON, CANADA; (2) Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, U.S.A.; (3) USDA Forest Service, Hardwood Tree Improvement and Regeneration Center, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S17

Benefits from high-throughput sequencing technology, such as 454 pyrosequencing, is most apparent for species with high societal or economic value but few genomic resources. However, it is questionable how well the sequencing of large numbers of short reads, for species with essentially no prior genome sequence information, will support SNP discovery. The focus of this research was to develop a method for identifying SNPs in a fungal genome using 454 pyrosequencing when no reference sequence is available. To discover SNPs, genomic DNA from eight isolates of *Sirococcus clavigignenti-juglandacearum* were bulked in one four-region sequencing run on a Roche 454 GS_FLX. This yielded 71 million total bases comprising 217 thousand reads, 80% of which collapsed into 16,125,754 bases in 30,339 contigs upon assembly. By aligning the reads from multiple isolates we detected 298 SNPs using Roche's gsMapper. However, with no reference sequence available, it was difficult to detect true polymorphisms. EagleView software was used to manually examine each contig that contained one or more putative SNPs. The program confirmed 45 of the original 298 putative SNPs. Of those 45 SNPs, 16 were validated using standard sequencing. This research provides the framework for the rapid and cost-effective discovery of SNP markers for non-model organisms and proves to be especially useful in the case of asexual or clonal fungi with limited genetic variability.

Reclassification of the butternut canker fungus, *Sirococcus clavigigenti-juglandacearum*, into the genus *Ophiognomonia*

K. D. BRODERS (1), G. J. Boland (1)
(1) University of Guelph, Guelph, ON, CANADA
Phytopathology 100:S17

Sirococcus clavigignenti-juglandacearum (*Sc-j*), which causes a canker disease on butternut, is largely responsible for the trees decline in the United States and Canada. The original description of the species was based largely on anamorphic characters as the teleomorph is unknown. Recent phylogenetic investigations found that *Sc-j* is not a member of the genus *Sirococcus*, and proper taxonomic classification is required. The aim of this study was to use sequence data to determine the phylogenetic placement of *Sc-j* within the *Gnomoniaceae*. Twenty-eight isolates of *Sc-j* from Ontario and across the eastern United States, in addition to representatives of the major lineages within the *Gnomoniaceae*, were included in the analysis. A portion of each of the genes coding for β-tubulin, actin, calmodulin, internal transcribed spacers 1 & 2, and the translation elongation factor 1-alpha were sequenced and evaluated. There were no differences in the sequences of the five genes among the isolates of *Sc-j* studied, providing evidence for a recent introduction into North America, followed by asexual reproduction and spread via conidia. The phylogenetic analyses clearly demonstrate the butternut canker fungus does not belong in the genus *Sirococcus*; and provided strong support (99% MP and 100% NJ bootstrap values) for its inclusion in the genus *Ophiognomonia*, thereby, supporting a reclassification of the fungus to *Ophiognomonia clavigignenti-juglandacearum.*

Revising the high temperature threshold for the Gubler-Thomas grape powdery mildew risk index

J. C. BROOME (1), E. K. Hand (1), P. Backup (1), C. N. Janousek (1), W. D. Gubler (1)
(1) University of California Davis, Davis, CA, U.S.A.
Phytopathology 100:S17

Powdery mildew, caused by *Erysiphe necator*, is an important disease of grapes. A temperature-driven disease risk model was developed by Gubler & Thomas (GT). Two years of detached leaf co-culture studies were conducted with single high temperature treatments at a range of durations and showed that *E. necator* continues to grow and reproduce in the lab at higher temperatures than previously reported. In 2009 we tested how consecutive, multiple heat treatments affected fungal growth parameters. We found that higher temperatures are increasingly lethal to the pathogen, reduce colony survival, and delay and reduce spore production. Temperature alone had a more pronounced effect than did the number of consecutive heat treatments (1, 2 or 3). Repeated consecutive exposures of 4 hrs at 36 and 38°C up to 3 days in a row resulted in less colony death and higher spore production, than one continuous exposure of 12 hrs at the same temperature. We are field testing several revisions of the GT model; raising the high temperature threshold and lengthening its duration from 35°C for 15 min, to 36°C and 38°C for 4 and 2 hrs, respectively. We adjusted how the index accounts for observed delays in fungal growth and reproduction. Future work will involve

integrating information on early season vineyard inoculum density and host varietal resistance into the model.

A tag-array minisequencing-based system for detecting and genetic fingerprinting *Wheat streak mosaic virus*: Implications for plant pathogen forensics

T. BROWN (1), U. Melcher (1), J. Fletcher (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S18

The U.S. Department of Homeland Security has identified several gaps in the security of the agricultural sector, indicating that one of the nation's largest economic contributors is vulnerable to a biological attack. Validated forensic tools that could be applied during a plant pathogen forensic investigation are needed for a balanced U.S. forensic capability. The goal of this project is to develop and validate a tool to simultaneously identify and forensically profile plant pathogens during the attribution of a biocrime. A tag-array minisequencing system was employed to profile a panel of single nucleotide polymorphisms (SNPs) from the model pathogen *Wheat streak mosaic virus*. Tailed primers terminating directly upstream from the SNPs were extended by one fluorescently labeled ddNMP during the minisequencing reactions. Products were subsequently hybridized to the tag array for detection and genotyping. Using synthetic targets, base misincorporation was found to be negligible and the mean values between technical replicates (n = 3) were indistinguishable using one-way ANOVA. The specificity of the assay has yet to be determined, however the primer design parameters are expected to yield strain specificity. The universality of this tool will provide a foundation for the development of similar systems for other plant and human pathogens. This tool also will be useful for rapid diagnostics and epidemiology studies during natural plant disease outbreaks.

Commercial extracts of the brown seaweed *Ascophyllum nodosum* and silicon reduce plant death due to *Fusarium solani* and increase yields of cucurbits

G. E. Brust (1), R. E. ROSS (2), J. Jayaraj (3)
(1) University of Maryland, Upper Marlboro, MD, U.S.A.; (2) Acadian Sea Plants LLC, Monroe, NC, U.S.A.; (3) The University of the West Indies, St. Augustine, TRINIDAD
Phytopathology 100:S18

Crop losses due to *Fusarium* spp. are important to cucurbit growers along with an increasing interest in natural ways to improve disease resistance. Extracts of the brown seaweed, *Ascophyllum nodosum* and products containing silicon have both been shown to promote disease resistance on many crops. In a 2008 watermelon trial located in Upper Marlboro, MD, *Fusarium solani* symptoms were suppressed by extracts of *Ascophyllum nodosum*. At the final rating, 30% of the watermelon plants were dead from this pathogen in the control plots vs. 10% in *Ascophyllum* extract treatments. A second study was implemented in 2009 on Gladiator Pumpkins. Calcium silicate and *Ascophyllum* seaweed extract were applied to pumpkins grown in a field known to have *Fusarium* spp. infected squash three years prior. At the final rating, 24.6% of the pumpkin plants were dead in the control plots vs. 19.2% in the silicon plots, 13.6% in the *Ascophyllum* extract treatment, and just 6.1% in the plots with both calcium silicate and *Ascophyllum* extract. These field studies were further supported by two greenhouse studies where applications *Ascophyllum* extract to cucumber plants reduced incidence of *Fusarium oxysporum* and enhanced the activities of plant defense-related enzymes including chitinase, β-1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and lipoxigenase as well as elevated levels of total phenols compared to the control.

The effects of soil steaming on the abundance of bacterial microflora in the rhizosphere and roots of Chrysanthemum

M. BURKETT-CADENA (1), E. van Santen (1), J. Kloepper (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S18

Soil steaming is a substitute for methyl bromide for managing soil-borne pathogens. In the cut-flower industry, negative effects of soil steaming have been reported as growers have noticed a reduction in flower size and increase in deleterious rhizobacteria in chrysanthemum. To examine the effects of steaming on the rhizosphere microflora, greenhouse experiments were conducted which documented changes in populations of total culturable bacteria, fluorescent pseudomonads, and aerobic spore forming bacteria (AEFB) after repeated steam applications. In the first cropping cycle, treatments included steamed soil, steamed soil + PGPR, and non-steamed soil. For the next cycle, half of the steamed soil was steamed, adding a fourth treatment. Rhizosphere bacteria were isolated, and populations determined by direct plate count. In the first cycle, bacterial populations from unsteamed soil were significantly lower than populations from steamed soil and steamed soil + PGPR. In the second cycle, steamed soil + PGPR retained higher populations of total bacteria and AEFB than unsteamed soil. Soil that was steamed twice had significantly higher populations of fluorescent pseudomonads than all other treatments, but had significantly lower populations of total bacteria than all other treatments. Populations in soil that was not resteamed for the second cycle differ from the control, suggesting that populations will not return to control levels even if steam is not reapplied.

Evaluation of inoculation methods for screening of rapeseed materials for resistance against *Sclerotinia sclerotiorum*

P. BURLAKOTI (1), V. Magnusson (2), W. D. Dai (2), L. E. del Rio Mendoza (1)
(1) Dept. Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.; (2) Dept. Plant Science, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S18

Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is an economic fungal disease affecting rapeseed (*Brassica napus* L) worldwide. Since expression of SSR symptoms shows much variability and the trait is quantitative in nature, reliable phenotypic evaluation methods for characterization of SSR resistance are needed. Six different inoculation methods were compared for their reliability to discriminate between *S. sclerotiorum*-resistant and susceptible materials. The methods were evaluated using two *S. sclerotiorum* isolates (WM031 and WM192) collected in North Dakota and 326 clonal plants derived from double haploid resistant (PI458940 x Ames 26628) and susceptible (Westar) *B. napus* materials. The methods involved mycelial inoculation on detached leaves and stems, petiole inoculation, straw inoculation, stem-piercing with toothpick, and mycelial spray. The experiment was conducted using a randomized complete block design with four replications and was repeated once. Detached materials were inoculated and incubated in laboratory at 16 hour light daily and 22°C temperature while inoculated plants were incubated in greenhouse at similar conditions. Detached stem and the petiole inoculation techniques produced the most consistent results and are considered more reliable to evaluate materials for their reaction to SSR.

Identification and analysis of *Fusarium verticillioides* genes differentially expressed during conidiation

A. M. BURNHAM (1), D. W. Brown (2), A. E. Glenn (3)
(1) Department of Plant Pathology, University of Georgia, Athens, GA, U.S.A.; (2) USDA, ARS, Bacterial Foodborne Pathogens & Mycology Research Unit, Peoria, IL, U.S.A.; (3) USDA, ARS, Toxicology & Mycotoxin Research Unit, Athens, GA, U.S.A.
Phytopathology 100:S18

Fusarium verticillioides, an endophytic maize pathogen, is the causal agent of diseases such as ear rot, seedling blight, and stalk rot, resulting in major economic losses in maize production. This fungus can also cause certain diseases in animals due to consumption of feed contaminated with fumonisin mycotoxins. *F. verticillioides* produces abundant microconidia in long chains from the apex of phialides. These conidia are essential for the infection of the crop. Upon mating two previously characterized conidia-producing strains, MRC 826 and NRRL 25029, spontaneous mutations occurred resulting in half of the progeny being unable to produce conidia. These mutants produced germ tube-like growths from the tips of phialides instead of normal enteroblastic production of conidia. Based on microarray data comparing MRC 826 (wild type) and AEG 3-A3-5 (aconidial mutant), thirteen candidate genes having at least 10-fold change in expression in the wild-type strain were chosen for further analysis. One of the thirteen, FVEG_10983, has been targeted for gene deletion because of its 72-fold change. FVEG_10983 has no homology to previously characterized proteins and no obvious protein domains. BLAST searches showed significant similarity only to other filamentous fungi including one homolog from *F. oxysporum* and three from *F. graminearum*. Further analysis of FVEG_10983 and the other genes may identify novel characteristics of sporulation in *F. verticillioides*.

Differential expression of putative polyketide biosynthetic gene clusters in *Fusarium verticillioides*

R. BUTCHKO (1), D. Brown (1), M. Busman (1), B. Tudzynski (2), P. Wiemann (2)
(1) USDA, Peoria, IL, U.S.A.; (2) Westfälische Wilhelms-Universität Münster Institut für Botanik, Münster, GERMANY
Phytopathology 100:S18

The maize pathogen *Fusarium verticillioides* can produce a number of polyketide derived secondary metabolites, including fumonisins. Fumonisins cause diseases in animals, and show epidemiological correlation with esophageal cancer and birth defects in humans. The *F. verticillioides* genome contains numerous polyketide synthase (PKS) genes, including *FUM1* which encodes the PKS required for fumonisin production. Only a small number of these PKS have been described with regard to the polyketide molecules they produce. Regulation of the expression and activity of fungal secondary

metabolite genes clusters can be effected by multiple factors including both pathway specific and nonspecific transcription factors. In *Aspergillus* species, LaeA has been shown to regulate the expression of secondary metabolite gene clusters at a higher hierarchical level, acting as a master regulator at the chromatin level. Here, the effects of the deletion of a putative *laeA* homolog in *F. verticillioides* are described. Toxin analysis revealed a decrease in fumonisin production, as well as other secondary metabolites. Microarray analysis indicates that the expression of the other putative secondary metabolite gene clusters is down- regulated in the *F. verticillioides laeA* deletion strain.

Exploring cover crop carbon sources for anaerobic soil disinfestation

D. M. Butler (1), E. N. ROSSKOPF (1), N. Burelle (1), J. Muramoto (2), C. Shennan (3)
(1) USDA ARS, Fort Pierce, FL, U.S.A.; (2) Center for Agroecology and Sustainable Food Systems, University of California, Santa Cruz, CA, U.S.A.; (3) Department of Environmental Studies and Center for Agroecology and Sustainable Food Systems, Santa Cruz, CA, U.S.A.
Phytopathology 100:S19

In Florida field trials, a raised-bed plastic mulch vegetable production system was developed using anaerobic soil disinfestation (ASD) for bell pepper/eggplant double crop production. Pathogen, weed, and nematode control was equivalent or better than methyl bromide. Molasses was used as the carbon source for supporting microbial generation of anaerobicity. To test warm season cover crops as replacements for molasses, a greenhouse trial was conducted using field soil in which tropical legumes and grasses and brassica species were grown and incorporated with or without composted broiler litter. Pathogen inoculum packets, yellow nutsedge tubers, and root-knot nematode eggs were introduced at cover crop incorporation. Anaerobicity (Eh) was monitored using oxidation-reduction probes and pathogen survival was assessed. All cover crops resulted in cumulative Eh values that were equal to the molasses treatment. Litter did not affect cumulative Eh in the greenhouse, but an interaction occurred between cover crop and litter with regard to survival of *Fusarium oxysporum* f. sp. *lycopersici* (Foxy) and *Sclerotium rolfsii* (SR). Foxy survival was reduced by more than 97% in all cover crops and the fallow treatment containing molasses when compared to the unamended control. Survival of SR was lowest in the molasses only treatment and in sorghum-sudan, pearl millet, and cowpea mixed with pearl millet or mustard-arugula with no litter.

Foliar fungicides reduce anthracnose top dieback of maize

E. BYAMUKAMA (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S19

Infection of maize by *Colletotrichum graminicola* may result in leaf blight, top dieback or stalk rot symptoms. Foliar applied fungicides have been reported to reduce stalk rot and top die back under high foliar disease severity. The effect of fungicide application on anthracnose top die back of maize was investigated at three locations in Iowa in 2009. At two locations, ISU Southeast Research Farm (SERF) and ISU Southwest Research Farm (SWRF), three fungicides, Headline (6oz/acre), Quilt (14oz/acre), and Stratego (4oz/acre), were applied at either tasseling, blister or milk growth stages to one hybrid. At Sorensen, Headline (6 oz/acre) was applied at tasseling to four hybrids with varying stalk rot susceptibility. No fungicide application served as the check. In all trials, the incidence of top die back at early dent, mean stalk rot severity at physiological maturity, yield, and grain moisture data were collected. Fungicide application significantly ($P < 0.0001$) reduced top die back incidence compared to the check at all the three sites. An application of a fungicide at VT reduced disease the greatest. No differences between fungicides were detected for top die back incidence. Fungicide treated plots had lower stalk rot severity at SERF and SWRF but not at Sorensen. There was a negative but significant correlation between grain yield and top back incidence ($r = –0.72$, $P = 0.04$) at Sorensen. Further work is needed to determine the effect of anthracnose top die back on yield of maize.

Assessment of new biomaterials for sample collection and nucleic acid recovery

D. J. CAASI (1), M. Arif (1), F. M. Ochoa-Corona (1)
(1) Oklahoma State University, Department of Entomology and Plant Pathology, National Institute for Microbial Forensic and Food & Agricultural Biosecurity (NIMFFAB), Stillwater, OK, U.S.A.
Phytopathology 100:S19

Collecting and archiving nucleic acids (NA) are key steps in detection and diagnosis when using PCR for agricultural biosecurity or microbial forensics applications. Paper-based technologies for collection offer several advantages, such as storage of NA at room temperature. However, the recovery of NA from this technology requires few steps and direct PCR is hampered by the residual paper matrix itself. We tested five biomaterials with soluble properties having different thicknesses (0.05 mm to 0.17 mm) for effectiveness in PCR amplification of NA. Scanning electron microscopy of pore spaces and crevices of the biomaterials either dry or wet, and with or without bacteria (*Pseudomonas syringae* pv. tomato) were retained after wetting. The absorbance of residual materials was measured in solution at 260 and 280 nm, and ranged from 0.014 to 0.034 O.D. at 260 nm, and from 0.013 to 0.030 O.D. at 280 nm. Optically measured residues were highest in the thickest biomaterial (0.17 mm) and lowest in the thinnest (0.05 mm). The five biomaterials were also blotted with DNA from *Pythium spinosum* or dsRNA from *Citrus leprosis virus* C and then subjected to PCR. Blotted DNA and RNA were successfully retrieved from all materials used; and residues were not inhibitory to PCR amplification. The assessed biomaterials have a potential application for streamlining both PCR-based protocols and NA storage during the collection and recovery of microbial NA.

New biocontrol strategy against the sexual fruiting bodies of grapevine powdery mildew

T. CAFFI (1), S. Legler (1), V. Rossi (1), L. Kiss (2), A. Pintye (2)
(1) Univ Cattolica del Sacro Cuore, Piacenza, ITALY; (2) Hungarian Academy of Science, Budapest, HUNGARY
Phytopathology 100:S19

Erisyphe necator is the casual agent of grapevine powdery mildew and its control is essentially based on chemicals. Potential BCAs (Bio Control Agents) were tested for their anti-powdery mildew effect and some commercial biofungicides were developed. Nevertheless, reports on their efficacy show that they cannot control *E. necator* efficiently. A new strategy was studied within the "BCA_grape project" (funded by EU Commision, 7th Framework Programme of Research) aimed at inserting biocontrol in an integrated disease management approach. This strategy includes i) new strains of the mycoparasite *Ampelomyces* spp.; and ii) new targets their application, i.e., the fruiting bodies of *E. necator* (chasmothecia). Ten different European *Ampelomyces* strains were selected for their ability in producing abundant conidia and germinating rapidly, under a wide range of environmental conditions. Ability of these strains of parasitizing chasmothecia was tested under controlled environmental conditions: they had different mycoparasitic activity, but all of them were more effective on young than mature chasmothecia. Late summer and fall applications of new *Ampelomyces* strains in vineyard (when first immature chasmothecia were formed) were able to significantly reduce the severity of ascosporic infections in the following spring and to delay the beginning of the epidemic compared to the untreated plots. A mathematical model was also developed to simulate chasmothecia maturation, as a tool for timing BCA application.

A test of taxonomic and biogeographic predictivity: Resistance to potato virus Y in wild relatives of the cultivated potato

X. CAI (1), D. Spooner (2), D. Halterman (2), A. Charkowski (3), R. Groves (4), S. Jansky (2)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) USDA/ARS, Madison, WI, U.S.A.; (3) University of Wisconsin-Madison, Plant Pathology, Madison, WI, U.S.A.; (4) University of Wisconsin-Madison, Entomology, Madison, WI, U.S.A.
Phytopathology 100:S19

A major justification for taxonomic research is its assumed ability to predict traits in a group for which the trait has been observed in a representative subset. In this study, we evaluated potato virus Y resistance using 135 accessions of potato to determine whether we can predict the distribution of resistance in wild *Solanum* species based on taxonomic or biogeographic data. Tremendous variation for PVY resistance was found within and among species. There is no consistent association between resistance and taxonomic series, clades, ploidy, and breeding system. However, the correlation coefficient between endosperm balance number (EBN) and PVY resistance was –0.22. The five species with the highest percentage of resistant plants were 1 EBN. A Chi-square test indicated that the 1 EBN species contain a higher percentage of resistant plants than expected. Our study identified new germplasm with resistance: *S. albornozii*, *S. andreanum*, *S. bukasovii*, *S. bulbocastanum*, *S. cardiophyllum*, *S. hjertingii*, *S. iopetalum*, *S. jamesii*, *S. kurtzianum*, *S. paucijugum*, *S. pinnatisectum*, and *S. schenckii*.A correlation between resistance and elevation at which the individuals were collected was high and significant. A Chi-square analysis revealed that there was a much higher than expected proportion of resistant plants in accessions collected at elevations below 2100m. This relationship may be related to the distribution of aphids, which act as vectors for PVY.

Genus *Alternaria* species as pathogens of *Vaccinium meridionale* in Colombia

C. Calderon (1), C. Socha (1), S. Restrepo (2), P. JIMENEZ (3)
(1) Bogotá, COLOMBIA; (2) Univ de Los Andes, Bogotá, COLOMBIA; (3) Univ Militar Nueva Granada, Bogotá, COLOMBIA
Phytopathology 100:S19

In previous studies, *Alternaria* sp. was identified as a pathogen of *Vaccinium meridionale*. Its attack induces a variable type of symptoms and is frequently recovered from the shoot. To know more about which species are involved in this pathosystem, different organs showing red spots, and necrotic spots were sampled in 3 different farms in Boyacá department, Colombia. After surface sterilization, the samples were cultured on PDA. *Alternaria* sp. was recovered from all of the samples. Taxonomic identification and Koch's postulates corroboration on detached organs and complete plants, were performed. Results showed that *A. alternata* and *A. tenuissima*, are the causal agent of disease. However, *A. alternata* induced red spots, that grew very slowly and showed a necrotic center. This species was more aggresive when a lesion was present on the tissue, then the invasion generated a fast growing necrotic area, and the conidiophores arranged in concentric circules. Meanwhile, *A. tenussima* showed a cottony mycelium, extended on the organ's surface and, suddenly, the tissue under the mycelium became necrotic. These results suggest that *A. alternata* is a mild pathogen, able to take advantage, agressively, of open courts in a more effective colonization. Differently, it seems clear that *A. tenuissima* is a strong pathogen able of penetrate the tissues in a direct way, without requiring open courts.

PGPR potential of Bacillus isolated from potato rhizosphere in the Andean highlands of Peru

P. CALVO-VELEZ (1), E. Ormeño-Orillo (2), E. Martínez-Romero (2), A. Oswald (3), D. Zuñiga-Davila (4)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Universidad Nacional Autónoma de México, Cuernavaca, MEXICO; (3) International Potato Center, Lima, PERU; (4) Universidad Nacional Agraria La Molina, Lima, PERU
Phytopathology 100:S20

Potato is an essential crop in Peru, contributing both to commercial and subsistence agriculture; however, there have been no comprehensive studies of its rhizosphere diversity. The aim of this investigation was to isolate, screen and identified bacteria of the genus Bacillus from the potato rhizosphere. Rhizosphere samples were collected from 21 fields in two highlands regions, representing several native varieties of potato. In a first screening of these isolates, their capacity to antagonize *Rhizoctonia solani* was evaluated. The strains which tested positive were further screened for *Fusarium solani* antagonism, IAA production, and phosphate solubilization. Finally a greenhouse trial was conducted and the strains were identified using molecular characterization with the 16s r RNA technique. We obtained a total of 63 isolates of *Bacillus* spp.; 43 (68%) of those showed positive control of *R. solani* ; and 91% of the selected strains also inhibited the growth of *F. solani.* IAA was produced at some level by 81% of the strains, and 58% solubilized tricalcium phosphate. In the greenhouse experiment a significant increase in number and dry weight of tubers, and total dry weight of the plant with 23 strains. Phylogenetic analysis revealed that the majority of the strains were *B. amyloliquefaciens*. Hence, the rhizosphere of native potatoes growing in the Andes is a rich source of antagonistic *Bacillus* spp. with potential future use as PGPR inoculants to improve potato production.

Sowing the Seeds of Science

A. R. Camp (1), H. W. Lange (1), S. Reiners (1), C. D. SMART (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 100:S20

Plant pathologists and other scientists at Cornell University's New York State Agricultural Experiment Station (NYSAES) are involved in an ongoing partnership with the Geneva City School District in Geneva, NY. The goals of this program are to (i) engage elementary aged students in a hands-on, inquiry-based study of science; (ii) generate awareness of how food is grown and where it comes from; and (iii) increase student exposure to agricultural sciences and related careers. Beginning in the spring of their third grade year, students are visited by Cornell faculty and learn about seeds, take a field trip to NYSAES to see the research that happens there, and help plant a vegetable and flower garden at their elementary school. During the summer, they have the opportunity to participate in a 5-week science camp run by school district faculty and scientists from Cornell. In the fall of their fourth grade year they share their new knowledge with the school community at a fall Harvest Festival. We have seen several positive outcomes from this program, including increased student knowledge, based on pre- and post-tests given during the Summer Science Camp; increased enrollment in local 4-H programs; and an increase in the proportion of students who pass the 4th grade science test mandated by New York State. In addition, this program helps plant pathologists and other scientists from Cornell foster good relationships with, and increase their impact in, their local community.

***Phytophthora capsici* in New York State: Resistance to mefenoxam and population structure**

A. R. CAMP (1), M. G. Milgroom (2), J. C. Meitz (3), A. McLeod (3), W. E. Fry (2), M. T. McGrath (4), H. R. Dillard (1), C. D. Smart (1)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Cornell University, Ithaca, NY, U.S.A.; (3) University of Stellenbosch, Stellenbosch, SOUTHWEST AFRICA; (4) Cornell University, Riverhead, NY, U.S.A.
Phytopathology 100:S20

More than 250 isolates of *Phytophthora capsici* were collected in 2006, 2007, and 2008 from sweet peppers, hot peppers, summer squash, winter squash, pumpkins, zucchini, tomatoes, and eggplants grown at 22 sites in four regions of New York State (western, central, Capital District, and Long Island). Isolates were assayed for mefenoxam resistance and assigned to multilocus genotypes (MLGs) based on mating type and five microsatellite loci. Mefenoxam-resistance was common in the Capital District and on Long Island, but not in western and central New York. Both A1 and A2 mating types were found at 12 of the 22 sites. At seven of the 11 sites from which at least ten isolates were sampled the ratio of A1 to A2 isolates did not differ significantly from 1:1. Of the 126 distinct MLGs identified, 117 were each restricted to only one of the 22 sites, and nine were detected at two or three sites each. From analysis of pairwise comparisons, it was learned that populations at nearly all sites were significantly different ($P \leq 0.05$) from each other. Much of the variation in the state-wide population could be attributed to differences either among regions or among sites. These results indicate that *P. capsici* in New York is highly diverse, but gene flow among different regions and fields is very restricted.

Impact of cropping sequence on diseases, nematodes, and yield of peanut, cotton, and corn in central Alabama

H. L. CAMPBELL (1), A. K. Hagan (1), K. L. Bowen (1), K. S. Lawrence (1), S. P. Nightengale (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) EV Smith Research Center, Plant Breeding Unit, Tallassee, AL, U.S.A.
Phytopathology 100:S20

A study was established at the Plant Breeding Unit, Tallassee, AL in 2003 to assess the impact of crop rotation on severity of diseases in peanut and corn as well as cotton root-knot nematode in corn, cotton, and peanut and to define the agronomic benefits or limitations of corn as a rotation partner with peanut and cotton. Peanuts were rated for leaf spot diseases, soil-borne diseases, and TSWV. Soil samples were taken for nematode assays shortly after each crop was harvested. In 2009, rotation and the Counter 15G soil insecticide treatment had a significant impact on corn yield and yield was significantly higher for the Counter 15G treated corn. Occurrence of CBR and peanut yield was significantly influenced by crop sequence but stem rot and leaf spot were not. TSWV levels were so low that only a few symptomatic plants were observed. CBR incidence was highest in plots maintained in continuous peanuts and yield was significantly lower than that of peanuts cropped behind one or two years of cotton or corn. Generally, yields for the one year out cropping sequence where cotton followed corn were higher compared with cotton followed by cotton and corn. Crop sequence had a significant impact on the yield of corn as well as on CBR and yield of peanut but not the severity of foliar diseases of corn or peanut.

Multiple effects of grafting on tomatoes and associated microbial communities

C. CAO (1), F. Baysal-Gurel (2), S. A. Miller (2), T. L. Graham (3), M. D. Kleinhenz (2), D. M. Francis (2), B. B. McSpadden Gardener (2)
(1) The Ohio State University, Wooster, OH, U.S.A.; (2) The Ohio State University, OARDC, Wooster, OH, U.S.A.; (3) The Ohio State University, Columbus, OH, U.S.A.
Phytopathology 100:S20

In order to determine if grafting affects the physiology, health, and associated microbial communities of tomato, rootstocks were left ungrafted, self-grafted or cross-grafted to cv. Celebrity before transplanting into the field in a randomized complete block design. Overall, ungrafted plants had more fresh and dry weight biomass than self-grafted regardless of rootstock ($P < 0.01$ for all comparisons). Nitrogen was 3–20% higher in leaves of self-grafted than ungrafted plants in 3 of 4 rootstocks tested ($P < 0.05$). Additionally, grafting was shown to influence levels of two secondary products present in apical leaves ($P < 0.05$ each). Comparisons of foliar disease incidence, i.e. late blight and Septoria leaf spot, revealed that self-grafted plants had less disease than ungrafted plants in three of four comparisons. In contrast, no significant variation in disease incidence was observed in cross-grafted plants, indicating that root stock genotype did not affect host resistance status. Terminal restriction fragment length polymorphism (T-RFLP) analyses of 16S and ITS sequences were performed to determine whether grafting or rootstock affected rhizosphere community structure. However, no clear patterns of grafting-induced changes in microbial community structure were observed. These results indicate that grafting-induced changes in host physiology, and not the root-associated microbial communities, can influence plant health several weeks after transplanting.

Fusarium stem blight of blueberry in Argentina

M. Caprara (1), E. R. WRIGHT (1), M. C. Rivera (1), B. A. Perez (2)
(1) Facultad de Agronomía, Universidad de Buenos Aires, Ciudad de Buenos Aires, ARGENTINA; (2) IMYZA-INTA, Hurlingham, Buenos Aires, ARGENTINA

Phytopathology 100:S21

In Argentina, Buenos Aires province is one of the major producing areas of blueberry (*Vaccinium corymbosum* L.). Disease surveys conducted in 2009 in the Indacochea area (Chivilcoy county) allowed to observe blueberry plants cv. O'Neal with dieback and stem blight. This disease affected 5% of the plants. Small pieces of the diseased branches were superficially disinfected and placed on 2% WA medium at room temperature. After purification, fungal morphological traits were studied in CLA, SNA and PDA. A pathogenicity test, with the *Fusarium* isolate obtained, was conducted by spraying a conidial suspension on the aerial part of unwounded and previously wounded one year-old blueberry plants cv. O'Neal. After 10 days, stem and leaf blight was recorded in unwounded and wounded plants but not in blueberry fruits. Studies are underway to confirm the section and species to which this *Fusarium* isolate may be considered.

***Phytophthora infestans* oospores: Production and viability in Colombia**

M. E. CÁRDENAS (1), M. C. Céspedes (1), A. J. Bernal (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA

Phytopathology 100:S21

Phytophthora infestans is the causal agent of the late blight disease in potato (*Solanum tuberosum*) and other members of the Solanaceae family (*S. phureja*, *S. lycopersicum*, *S. betaceum*, *S. melongena*, *S. quitoense* and *Physalis peruviana* among others). In Colombia, the incomes of many people, mainly farmers, depend on most of these crops, mainly potato, tree tomato and cape gooseberry. Recently, the A2 mating type was reported for the first time in this country and opened the possibility to a higher genetic variation in the *P. infestans* population that could lead to changes in its fungicides susceptibility patterns, an increased virulence or a broadening of host range. However, last year, a *P. infestans* population survey showed that in Colombia this pathogen is clonal despite the presence of the A2 mating type. In order to understand the population dynamics of this pathogen in Colombia crosses between A1 mating type from different hosts and the Colombian A2 mating type from cape gooseberry were tested for the production, viability and germination of oospores. Apparently, the host adaptation is not an explanation to the reduced c.f. inexistent sexual reproduction of this pathogen in Colombia. Besides the isolation low frequency of the A2 mating type a post-mating incompatibility is suggested according to the low viability and therefore the germination percentages obtained.

Effects of pH on genes involved in oxalic acid production in the brown rot fungus *Postia placenta*

B. CARLSON (1), J. Jellison (1)
(1) University of Maine, Orono, ME, U.S.A.

Phytopathology 100:S21

Brown rot fungi are the organisms primarily responsible for the biodegradation of soft, in-service wood. During the decay process, brown rot decay fungi such as *Postia placenta* release large amounts oxalic acid (pKa1 = 1.27, pKa2 = 4.28) which rapidly dissociates into H+ and oxalate ($C_2O_4^{2-}$). To characterize the relationship between glucose consumption, oxalic acid production and pH, a time course study was run over 21 days on *P. placenta* grown in 50 ml of modified Highley's media. Shake cultures with 1 mM asparagine and ammonium-L-tartrate and 5% glucose were harvested after 1, 3, 5, 7, 13, 17, 19, 22 days following inoculation and were analyzed for glucose, oxalic acid, pH, and biomass. As available glucose in culture decreased, pH decreased from 4.73 ± 0.03 to 2.81 ± 0.06 and oxalic acid increased from below detection limits to 0.51 ± 0.10 mg/ml. To determine the effect pH has on gene expression of enzymes involved in oxalic acid synthesis, static cultures were grown and pH was adjusted to 7 with 2M NaOH. 17 hours after treatment, the pH of the treated culture dropped and mycelia were harvested for real time PCR. Preliminary results (n = 1) show a 9.4 fold increase in glyoxylate oxidase but no significant change in oxalate decarboxylase, oxaloacetase, isocitrate lyase gene expression.

Monitoring latent *Colletotrichum acutatum* infections in strawberry using a bioassay and real time PCR in organic and conventional systems

M. E. CARNES (1), M. Rahman (1), F. J. Louws (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.

Phytopathology 100:S21

Strawberry anthracnose fruit rot, caused by *C. acutatum* is an economically important disease in the southeastern United States. Latent infections (LI) can lead to wide-spread pathogen dispersal due to the shipment of infected plant material. In 2009, strawberry leaves were sampled from 40 plants weekly at an organic and conventional farm and LI was evaluated using a paraquat dip bioassay. LI incidence was 15% in leaves sampled at the organic farm on 10 March, and increased to 67% by 1 May. In contrast, at the conventional farm where fungicide was applied as a preventative measure, LI incidence reduced throughout the monitoring period from 20% on 17 Mar to 5% by 23 April. The paraquat assay is effective, but requires handling of toxic chemicals and a 7 day incubation period. A quantitative PCR (qPCR) protocol was developed for rapid and more sensitive screening. During the 2009–10 growing season, infection incidence was compared using qPCR and paraquat dip assays on leaves sampled from the same plants. Compared to the paraquat assay, utilization of the qPCR TaqMan protocol increased detection of latent infections by 39%. Equally efficient results were obtained using a SYBR green protocol. Compared to very young or old leaves, greater sensitivity was observed by using middle-aged leaves to evaluate LI on foliage. Modification of the qPCR protocol for high throughput screening of LI incidence may provide an additional tool in integrated disease management strategies.

Additional sources of broad-spectrum resistance to *Puccinia coronata* f. sp. *avenae* in Canadian accessions of *Avena barbata*

M. CARSON (1)
(1) USDA ARS, St. Paul, MN, U.S.A.

Phytopathology 100:S21

Crown rust (*Puccinia coronata* f. sp. *avenae*) is the most damaging disease of oat and race-specific seedling (*Pc*) genes for resistance have been the primary means of control. As resistance genes have been deployed in cultivars, corresponding virulence in the crown rust population increased rapidly, such that the effective lifespan of a resistant cultivar in the U.S. is now five years or less. Introgression of resistance from diploid and tetraploid *Avena* species into hexaploid oat has been difficult due to differences in ploidy levels and the lack of pairing of homeologous chromosomes. The wild tetraploid *A. barbata* has been a source of powdery mildew and stem rust resistance in cultivated oat, but has largely been unexploited for crown rust resistance. Tests of 1099 *A. barbata* accessions from the Canadian Plant Gene Resources Center, not represented in the USDA collection, revealed that 11.4% were at least moderately resistant at the seedling and adult plant stages when tested with a highly diverse bulk inoculum derived from the St. Paul buckthorn nursery. Eighteen accessions were rated as highly resistant or a mix of highly resistant and resistant plants in both seedling and adult plant tests. Three accessions displayed a unique 'blotchy' resistant reaction as adult plants. Resistant accessions were found from throughout much of the natural range of *A. barbata*, but the Western Mediterranean and Lebanon had the highest frequency of accessions with broad-spectrum resistance.

Development of a quantitative real-time PCR protocol for the analysis of early colonization of sugarcane plantlets with *Leifsonia xyli* subsp. *xyli*

G. Carvalho (1), R. S. de Faria (1), F. R. Oliveira (1), A. T. Ramos (1), C. B. Monteiro-Vitorello (1), L. E. CAMARGO (1)
(1) University of São Paulo, Piracicaba, BRAZIL

Phytopathology 100:S21

The bacterium *Leifsonia xyli* subsp. *xyli* (Lxx) causes the ratoon stunting disease (RSD), one of the most important diseases of sugarcane worldwide. The objective of this study was to develop a quantitative real time PCR assay and use it to study the dynamics of early colonization of sugarcane with Lxx in a susceptible and a resistant variety. Primers specific for Lxx were designed based on its available genome sequence and used to quantify Lxx in leaves of young plants. *In vitro* grown plantlets were transferred to pots and inoculated 60 days after by cutting them just above the apical meristem and placing a 30 µL volume of a liquid culture (OD = 0,8) on the cut surface. Bacterial populations were estimated in leaf DNA extracts 10, 20, 40 and 80 days after inoculation. The results indicated a rapid *in planta* growth of Lxx in the susceptible variety that sharply contrasts with its fastidious behavior in artificial medium. This suggested that a sizeable colonization of sugarcane tissues occurs well before the manifestation of external symptoms, which happen at least 9 months after inoculation. In the resistant variety, no significant changes in bacterial titers were detected over time. Further studies should be pursued to determine if this rapid inoculation and quantification method reflects the level of resistance displayed by sugarcane varieties under field conditions. If so, the methodology described could be used as a rapid screening method to select resistant genotypes.

Isolation and molecular identification of *Fusarium oxysporum* f. sp. *vasinfectum* race 2 present in Alabama cotton

J. D. CASTILLO (1), K. S. Lawrence (1), L. F. Cruz (1), K. M. Glass (1)
(1) Auburn University, Auburn, AL, U.S.A.

Phytopathology 100:S21

Fusarium oxysporum f. sp. *vasinfectum* (FOV) causes Fusarium wilt of cotton and was first reported in Alabama by Atkinson in 1892. Currently, eight races of FOV have been described around the world. Historically races 1 and 2 have

been reported in the U.S., but recent studies in the southeastern U.S. reveal the presence of four novel genotypes, and the presence of races 3 and 8 outside of California. Therefore, the objective of this study was to identify the races of FOV present in the Auburn University Fusarium wilt research test field. FOV from thirty cotton lines with varying root-knot nematode susceptibility were isolated from symptomatic cotton plants and confirmed as FOV based on morphology. Race was determined based on the sequences of three different genes and enzyme restriction digestion analysis previously reported. Partial sequences of translational elongation factor (EF-1α), phosphate permease (PHO), and beta-tubulin (BT) genes were analyzed, and a maximum parsimony tree was generated from these gene sequences. Additionally, a restriction enzyme digestion analysis of the intergenic spacer (IGS) region of nuclear rDNA was performed. Fragments amplified were 561, 404, and 810 bp for EF-1α, PHO, and BT, respectively. Preliminary results confirm that the FOV race 2 is present in Alabama, and the FOV isolates fit in the Lineage II (composed by race 1, 2, and 6) in the maximum parsimony tree.

Multilocus analysis of *Rhizoctonia solani* and related species associated with rice sheath blight in Arkansas

V. L. CASTROAGUDIN (1), R. D. Cartwright (1), J. C. Correll (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S22

Sheath blight (SB) is one of the most important diseases of rice in the southern United States and *Rhizoctonia solani* AG 1-IA is considered the primary causal agent. However, *R. solani* AG 11, *R. oryzae*, *R. oryzae-sativae*, *R. zeae*, and *Sclerotium hydrophilum* can also be isolated from rice with SB symptoms. More information about the molecular diversity of *R. solani* and related species affecting rice is needed. In this study, the phylogenetic relationship of a diverse collection of *Rhizoctonia* spp. from rice in Arkansas was examined by a multilocus sequencing approach, simple sequence repeats (SSR), and universal primed PCR (UP-PCR) analysis. For multilocus-sequence based analysis, the targeted loci were the internal transcribed spacer (ITS) region of ribosomal DNA, cytochrome oxidase 1, the first and second largest subunits of RNA polymerase II, beta-tubulin, and six anonymous nuclear markers. The data analysis indicates the presence of inter- and intra-specific diversity at the nuclear loci examined. Limited sequence variation was observed within the ITS region of *R. solani* AG 1-IA. The population diversity of AG 1-IA and AG 11 isolates also was examined with SSR and UP-PCR markers, and isolates of *R. solani* AG 1-IA could be distinguished into eight and thirteen haplotypes, respectively.

A comparison of standard and high-fidelity PCR in the detection of *Sclerotium rolfsii* and a *Dickeya* sp. from *Phalaenopsis* orchids

R. A. Cating (1), M. A. Hoy (2), A. J. PALMATEER (1)
(1) University of Florida, Homestead, FL, U.S.A.; (2) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S22

The polymerase chain reaction (PCR) has become widely used in phylogenetic and genomic analyses, plant-disease diagnoses, and to examine pathogen diversity. However, standard PCR usually does not amplify sequences of more than 5 kb and can be inhibited by plant cellular contents including host genomic DNA when used for the detection of plant pathogens directly from plant tissue. The high-fidelity PCR has been used to detect a number of microbes while in the presence of host genomic DNA and is more efficient than the standard PCR. In this study, high-fidelity and standard PCRs were used to detect *S. rolfsii* and a *Dickeya* sp. directly from inoculated orchids. The high-fidelity PCR could detect the presence of the pathogen in all inoculated plants, while the standard PCR could detect only the positive controls. These results indicate that the high-fidelity PCR may enable a dramatic improvement in the detection of a wide range of plant pathogens, especially when low template concentration, contaminating or competitor DNA, or amplification inhibitors exist in a given sample.

Molecular and morphological characterization of a *Phytophthora infestans* population in the Colombian Andean region

M. C. CÉSPEDES (1), M. E. Cárdenas (1), A. M. Vargas (1), A. J. Bernal (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA
Phytopathology 100:S22

Late blight caused by *Phytophthora infestans* is one of the most devastating diseases of potato in the world. This pathogen can also attack economically important crops in Colombia such as Cape gooseberry, tomato and naranjilla. To establish an effective control to prevent this disease, it is necessary to know the genetic structure of the population. In the present study we collected 23 isolates from different hosts in two departments, Cundinamarca and Boyacá. The objectives of the study were i) to characterize the resistance level of the isolates to Mefenoxam ii) to establish the baseline of sensitivity of the isolates to two fungicides (Mefenoxam and Cymoxanil) iii) to determine the physiological races present in the population and iv) to characterize the molecular diversity of the isolates using one avirulence gene (*Avr3a*), the β-tubulin gene and two single copy genes with an RXLR motif. We found that 52% of the population showed sensitivity to mefenoxam. Results obtained from the molecular diversity suggested that the population is clonal in this Colombian region. These results along with the current study of the physiological races provide new insights in the characterization of this population. The gather information will help in the development of durable management strategies for the future, managing fungicide resistance and contributing for wider studies on global diversity.

***Cmm*-tomato interactions: Visualization during infection, biofilm formation and epiphytic fitness**

L. CHALUPOWICZ (1), O. Dror (2), M. Cohen-Kandli (2), R. Eichenlaub (3), E. Zellermann (3), K. Gartemann (3), G. Sessa (4), I. Barash (4), S. Manulis-Sasson (2)
(1) Tel Aviv University, Ramat Aviv, ISRAEL; (2) ARO, The Volcani Center, Beit Dagan, ISRAEL; (3) University of Bielefeld, Bielefeld, GERMANY; (4) Tel-Aviv University, Tel Aviv, ISRAEL
Phytopathology 100:S22

The Gram-positive actinomycete *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the causal agent of tomato wilt disease, an economically important disease worldwide. Pathogenicity of *Cmm* is associated with two plasmids, pCM1 and pCM2 and a *chp/tomA* pathogenicity island. *Cmm* colonization of tomato plant by microscopic methods, as well as epiphytic fitness on tomato leaves and biofilm formation were examined. Confocal laser scanning microscopy revealed that GFP-labeled *Cmm* extensively colonized the xylem vessels with preferential attachment to spiral rings and tracheary elements of wilted leaves. Scanning electron imaging showed aggregates of *Cmm* cells between the spiral elements at 7days post-inoculation, while after 15 days *Cmm* densely colonized the whole xylem vessels. Moreover, *Cmm* cells were observed on remnants of cell wall which appeared perforated and might result from cell wall degrading enzymes. Crystal violet staining assay showed that biofilm formation of *Cmm* grown in xylem sap was significantly higher as compared to Luria Broth and minimal medium by 2.5 and 1.5 fold, respectively. In addition of being an efficient endophyte, *Cmm* showed high epiphytic fitness on tomato as compared to bean leaves. These results were supported by the observation that GFP-labeled *Cmm* cells densely colonized tomato leaf surfaces. This is the first study in which GFP-labeled *Cmm* was employed to demonstrate endophytic and epiphytic characteristics of this phytopathogen.

Description and evaluation of an education and outreach program for best management of *Ralstonia solanacearum* race 3 biovar 2

P. G. CHAMPOISEAU (1), J. B. Jones (1), C. Allen (2), T. M. Momol (3)
(1) University of Florida, Department of Plant Pathology, Gainesville, FL, U.S.A.; (2) University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI, U.S.A.; (3) University of Florida, District Directors Office, Gainesville, FL, U.S.A.
Phytopathology 100:S22

Ralstonia solanacearum race 3 biovar 2 (R3b2) is considered a serious quarantine pest in Canada and Europe and is listed as a Select Agent plant pathogen in the United States, where it is subject to the strictest biosecurity regulations. Due to quarantine regulations, it is critical to prevent re-introduction and possible spread of this pathogen in the U.S., and this primarily involves exclusion, early detection, and unambiguous identification of the pathogen; it also requires preparedness and effective training of official regulators, diagnosticians, industry members, and other individuals responsible for first detection and response to a possible R3b2 discovery in the U.S. To achieve this objective we have developed a 3-year integrated education and outreach program, as part of a USDA-founded project, to target multiple audiences in the U.S. This program included participation to multiple meetings and conferences, use of current web-based technologies, and development of e-learning modules for delivery of up-to-date educational materials. Along with the presentation of these new educational materials, we describe the development and use of various evaluation tools to assess program effectiveness. Among these tools, monitored access of our Ralstonia/bacterial wilt-dedicated website shows that stakeholders from diverse organizations both within and outside the U.S. regularly use this resource to obtain updated and accurate information on R3b2 and bacterial wilt disease management.

Possible functions of light-induced proteins in cercosporin biosynthesis by *Cercospora kikuchii*

A. K. CHANDA (1), Z. Chen (1), R. W. Schneider (1)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.
Phytopathology 100:S22

Cercospora kikuchii is the causal agent of leaf blight and purple seed stain of soybean. *C. kikuchii* has established in the southern United States and caused

significant yield reduction in soybean. The pathogenicity of *C. kikuchii* has been attributed to a polyketide photosensitizer, cercosporin. Cercosporin is a non-host specific toxin produced by plant pathogens that belong to genus *Cercospora*. There has been a great interest in understanding cercosporin biosynthesis by many researchers, which can help in managing diseases caused by *Cercospora* species and also to minimize the loss from the diseases. We utilized two-dimensional protein gel electrophoresis (2DGE) to identify possible proteins involved in cercosporin biosynthesis by comparing protein profiles of *C. kikuchii* grown under cercosporin-favoring (light) and cercosporin-suppressing (dark) conditions. We identified and sequenced several proteins that were differentially expressed under light and dark. The light up-regulated proteins showed high homology to proteins like hydroxynaphthalene reducatse, S-adenosylmethionine synthetase, as well as to the expressed sequence tags (ESTs) from *Cercospora zeae-maydis*. Their corresponding genes have been cloned to determine their roles in cercosporin biosynthesis and pathogenicity of *C. kikuchii*.

A survey of fungicide resistance in the *Venturia inaequalis* populations of Indiana and Michigan

K. S. CHAPMAN (1), G. Sundin (2), J. Beckerman (1)
(1) Purdue University, West Lafayette, IN, U.S.A.; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S23

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. The presence of resistance to all major fungicides used for apple scab management leaves growers with fewer options for control and a higher risk for crop loss.

Molecular approaches for unraveling phytopathogenic fungi – *Macrophomina phaseolina* in cluster bean

A. CHAUDHURY (1)
(1) Department of Bio & Nano Technology, Giuru Jambheshwar University of Science & Technology, Hisar, Haryana, INDIA
Phytopathology 100:S23

Soil borne phytopathogenic fungus *Macrophomina phaseolina* is prevalent in over 500 crops species, especially, cluster bean, cotton, okra, soybean, in India, Brazil, United Kingdom, Australia, North and South America, Africa and parts of Europe by causing Charcoal rot disease is a major constraint for higher yields. Genetic and phenotypic characterization of various isolates obtained from several host plants using conventional techniques, PCR based molecular markers: RAPD, PCR–RFLP of rDNA ITS region, Microsatellite primed PCR and Rep-PCR conducted for evaluation of genetic diversity as an initial step towards understanding population structure of charcoal rot pathogen. Results reveal that it is a rapid, inexpensive technique, highly reproducible and almost as discriminatory as MSP-PCR for genotyping *M. phaseolina* isolates. It is quite suitable for understanding disease epidemiology at molecular level, suggesting, thereby, that it is a robust technique employed for genotypic and phylogenetic studies for determining taxonomical diversity of commercially important cluster bean. Experiments on optical properties of *Macrophomina phaseolina* impregnated Sol-gel-derived silica matrices revealed that it is quite rapid as compared to conventional calorimetric methods. Now efforts are underway for developing SCAR as a diagnostic tool for detection of *M. phaseolina.* Data presented here will serve as platform in designing strategies for breeding program, epidemiological and other taxonomic studies in future.

Systemic acquired resistance for reducing bacterial wilt severity on annual bluegrass

A. CHAVES (1), N. A. Mitkowski (1)
(1) University of Rhode Island, Kingston, RI, U.S.A.
Phytopathology 100:S23

Annual bluegrass (*Poa annua* L.) is a common grass species found on golf courses. Although a weed, it is managed as the primary turf on many putting greens in the Northeast. *Poa annua* is susceptible to numerous diseases including bacterial wilt, caused by *Xanthomonas translucens* pv. *poae*. The bacteria multiply in xylem vessels, causing wilting and necrosis. Few control options exist for this disease. Copper compounds can be used but have a high level of phytotoxicity. Antibiotics are not labeled for use on golf courses. We have studied induced resistance as a possible control for this disease. Trials were undertaken to study the effects of exogenous applications of salicylic acid, 2,6-dichloroisonicotinic acid (INA), Actigard® (acibenzolar-S-methyl), Aliette® (fosetyl-aluminum), beta-amino butyric acid (BABA), Messenger® (purified harpin protein), and dihydrogen potassium phosphate (K_2HPO_4). These chemicals have been shown to induce resistance in other plant species by mimicking systemic acquired resistance. Our results demonstrate that INA, SA, and Actigard are the most effective in inducing resistance, frequently increasing survival to between 30 and 60% in greenhouse trials conducted under high humidity (> 75%) and high temperatures (25°C). Some plant survival was also seen using Aliette, K_2HPO_4, and BABA but at lower levels. Harpin appears to be minimally effective. No previous work has been done on the efficacy of induced resistance in controlling bacterial wilt on annual bluegrass.

Functional analysis suggests evolutionary conservation of Pto and Rsb *Pseudomonas* resistance phenotypes between tomato and potato

Y. CHEN (1), M. Stolz (2), K. Neu (3), D. Halterman (3)
(1) Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) Memorial High School, Madison, WI, U.S.A.; (3) U.S. Department of Agriculture-Agriculture Research Service, Vegetable Crops Research Unit, Madison, WI, U.S.A.
Phytopathology 100:S23

Pathogen effector, AvrPtoB, from *Pseudomonas syringae* pv. *tomato*, has two distinct avirulence determinants. One contains the first 307 amino acids ($AvrPtoB_{1-307}$), which is recognized by the Pto kinase and leads to hypersensitive response (HR). The other contains an additional 80 amino acids ($AvrPtoB_{1-387}$) that are recognized by the Fen kinase and also leads to the HR. The C-terminal region of AvrPtoB has E3 ligase activity, ubiquitinating the Fen kinase, leading to its degradation and thus suppressing host defense. Interestingly, although the Pto and Fen kinases share 80% of amino acid identity, Pto is resistant to AvrPtoB-mediated degradation. A host resistance phenotype, Rsb (Resistance suppressed by AvrPtoB C-terminus), which is conferred by Fen, occurs more frequently than Pto-like resistance in wild species of tomato. In our study, we tested multiple individuals of potato from 10 different wild species by *Agrobacterium* infiltration. We found that Rsb was also very common among wild potato species and we identified Pto-like resistance in two different species. Interestingly, we also observed several different recognition patterns of truncated and full-length AvrPtoB. Pto-like genes from these wild species of potato have been cloned and sequenced with the expectation that elucidation of interactions between Pto-like genes from potato and AvrPtoB will help us understand how Pto-mediated resistance evolved in the arms race between host and pathogen.

High-throughput detection of *Sclerotinia sclerotiorum* from oilseed rape by Taqman quantitative realtime PCR

C. CHEN (1), W. Zhao (1), M. Zhou (1), J. Wang (1)
(1) Nanjing Agricultural University, Nanjing, PRC PEOPLES REP OF CHINA
Phytopathology 100:S23

Stem rot of oilseed rape is caused by *Sclerotinia sclerotiorum*, one of the most damaging pathogens. In this study, beta-tubulin from *S. sclerotiorum* was applied to develop a Taqman quantitative realtime PCR to detect populations of the pathogen. One Taqman probe (5′-Rox-CGA GAA CTC TGA CGAGAC CTT CTG TA-Tamra-3′) and one primer pair TYF (5′- ATA TAACGCTACTCTCTCTGTTC -3′)/TYR (5′- AGCCAACTTTCGG AGATTTG-3′) were developed and synthized according to the specificity of the beta-tubulin gene (GenBank access number AY312374) of *S. sclerotiorum*. Amplifications were conducted in 25 µl volumes containing d_2H_2O 10 µL, buffer (10x) 2.5 µl, $MgCl_2$ (25mM) 5 µL, dNTP (10 µM) 2 µL, TYF (10 µM) 0.5 µL, TYR (10 µM) 0.5 µL, fluorescent probe (10 µM) 2 µL, genome DNA from sclerotia (50 µg/mL) 2.0 µL, Taq DNA ploymerase (5 u/µL) 0.5 µL. The PCR amplifications were performed using the following parameters: an initial pre-heat for 10 sec at 95°C, followed by 40 cycles at 95°C for 15 s, denaturation at 56°C for 60 s, extension at 72°C for 30 s. Stardard curve equation Y = -3.30X+24.30 (R^2 = 0.995) was set up to detect the population of *S. sclerotiorum*. The technique appears suitable for the epidemiological studies such as high-throughout detection of *S. sclerotiorum* populations from diseased stems, leaves and petals of oil rape. All the procedure of detection may be finished within 7 h. The real-time PCR assay

was developed in this study could help growers make a timely decision on fungicide application.

Guangdong (China) and Florida (U.S.) populations of "Candidatus Liberibacter asiaticus" distinguished by a genomic locus with short tandem repeats

J. CHEN (1), X. Deng (2), X. Sun (3), D. Jones (3), M. Irey (4), E. Civerolo (5)
(1) USDA ARS PWA, Parlier, CA, U.S.A.; (2) South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA; (3) Division of Plant Industry, Department of Agriculture and Consumer Services, Gainesville, FL, U.S.A.; (4) US Sugar Corp./S. Gardens Citrus, Clewiston, FL, U.S.A.; (5) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 100:S24

Huanglongbing (HLB, yellow shoot disease) is a highly destructive disease that threatens citrus production worldwide. The disease was first observed in Guangdong, P. R. China over 100 years ago, and was found in Florida, U.S.A. in 2005. "Candidatus Liberibacter asiaticus" has been associated with HLB. Understanding the global epidemiology of HLB is important for management of the disease. In this study, we identified a genetic marker containing small tandem repeats in the genome of "Ca. L. asiaticus" and comparatively analyzed the tandem repeat numbers (TRNs) in bacterial populations from Guangdong and Florida. The Guangdong population consisted predominately of strains with TRN of 7 (TRN7) at a frequency of 47.6%. The Florida population consisted predominately of strains with TRN of 5 (TRN5) at a frequency of 84.4%. TRNs ranged from 3 to 16. The apparent absence of TRN of 9, 10, 11, and 12 separated the bacterial strains into two groups: TRN less than 10 (TRN<10) and TRN greater than 10 (TRN>10). In Florida, TRN<10 strains (103/109 or 94.5%) were widely distributed in all HLB-affected counties. TRN>10 strains (6/109 or 5.5%) were found in central Florida. This is the first report documenting the differentiation of "Ca. L. asiaticus" populations between Asia and North America and the possible presence of two differentially distributed genotypes of "Ca. L. asiaticus" in Florida.

Evaluation of tandem repeat polymorphisms between two pathogenically similar strains of Xylella fastidiosa from almond and grape in California

J. CHEN (1)
(1) USDA ARS PWA, Parlier, CA, U.S.A.
Phytopathology 100:S24

Whole genome tandem repeat polymorphisms were evaluated between two closely related Xylella fastidiosa strains, M23 and Temecula1, both cause almond leaf scorch disease (ALSD) and grape Pierce's disease (PD) in California. Strain M23 was isolated from almond and the genome was sequenced in this study. Strain Temecula1 was originally isolated from grape and its genome was sequenced previously. Among the 48 identified tandem repeat (TR) loci evaluated by sequence similarity flanking the TRs, two were unique to strain M23, six were unique to strain Temecula1, and 40 were shared by both bacterial strains with a similarity of >70%. Yet, the two strains differ in TR numbers (TRNs) in all shared loci. Eight shared loci were selected to evaluate TRN variation using additional 10 X. fastidiosa strains. Results from our analyses indicate that TRN comparison could be highly powerful for discriminating closely related bacterial strains. However, careful selection and evaluation of TR loci is critical in order to avoid over estimating of bacterial strain genetic diversity.

The mechanisms associated with cellular resistance of calamondin and kumquat to citrus canker caused by *Xanthomonas anxonopodis* pv. *citri*

B. CHEN (1), L. Wang (2), K. Chung (3), M. Lee (1)
(1) Department of Plant Pathology, National Chung Hsing University, Taichung, TAIWAN; (2) Plant Protection Department, Chiayi Agricultural Experiment Branch, Agricultural Research Institute, Council of Agriculture, Chiayi, TAIWAN; (3) Citrus Research and Education Center, Institute of Food and Agricultural Sciences (IFAS), University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S24

Citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*), is one of the most severe diseases on citrus in Taiwan. It has long been known that calamondin (× *Citrofortunella microcarpa*) and kumquat (*Fortunella* spp.) are highly tolerant to *Xac* compared to other citrus cultivars. Molecular and physiological approaches were used to explore possible mechanisms attributed to *Xac* resistance. Pathogenicity assays on leaves wounded prior to spray inoculation revealed that *Xac* induced flatter necrotic lesions on calamondin and kumquat, but promoted characteristic canker lesions with raised and corky appearance on the susceptible variety, Mexican lime (*Citrus aurantifolia*). When inoculated by infiltration, *Xac* propagated at lower rate and magnitude in kumquat compared to Mexican lime or calmondin. Further analysis revealed that the peel, but not leaf, extracts from calamondin or kumquat displayed inhibitory effects to *Xac* as assayed in culture, implicating the presence of preformed compounds. The levels of antioxidant enzymes were very different in calamondin, kumquat, or Mexican lime before or after *Xac* challenge. In comparison with Mexican lime or calamondin, leaf extracts from kumquat had lower superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPx). However, these enzymatic activities in kumquat promptly increased after inoculation. Thus, our results suggest that both preformed and induced defense compounds of kumquat may have an important role in cellular resistance to *Xac*.

Characterization of a putative *Ustilago maydis* pathogenesis gene, *Upe*

H. K. CHEUNG (1)
(1) Trent University, Peterborough, ON, CANADA
Phytopathology 100:S24

Genome annotation of the corn smut pathogen *Ustilago maydis* revealed a large number of genes potentially involved in the completion of meiosis. This pathogen requires growth in the plant to become competent to undergo meiosis and therefore we hypothesize that signals received from the host plant stimulate *U. maydis* meiotic development. One such gene, an ortholog of the transcription regulator *Ume6*, was investigated. Deletion of this gene did not result in a discernable haploid cell phenotype. However infection with compatible deletion strains resulted in increased pathogenesis and earlier onset of disease symptoms. Teliospores that were produced from these infections germinated and completed meiosis in a manner indistinguishable from wild type cells. This indicates that this *Ume6* ortholog is not required for meiosis; however it suggests a role in pathogenic development. Since the structure of the gene indicates that it is likely a transcription factor we have named the gene *Upe* for unregulated pathogenesis gene expression. The pattern of pathogenesis in the *Upe* deletion strains is consistent with *Upe* being either a repressor of pathogenesis genes or an activator of genes that suppress the host plant response. Further mutant strains in which the expression of *Upe* is altered have been created and the impact of these mutants on haploid cell growth and pathogenic development will be presented along with progress on determining variation in gene expression responses.

Environmental regulation of stomate-based defense against bacterial infection in *Arabidopsis*

R. CHITRAKAR (1), M. Melotto (1)
(1) University of Texas at Arlington, Arlington, TX, U.S.A.
Phytopathology 100:S24

Stomata are natural openings in the plant epidermis responsible for gas exchange between plant interior and environment. They are formed by a pair of guard cells, which are able to close the stomatal pore in response to a number of external factors including light intensity, carbon dioxide concentration, and relative humidity (RH). The stomatal pore is also the main route for pathogen entry into leaves, a crucial step for disease development. Recent studies have unveiled that closure of the pore is effective in preventing bacterial disease in *Arabidopsis* plants and the successful plant pathogen *Pseudomonas syringae* pv. tomato (*Pst*) DC3000 is able to re-open stomata by producing the phytotoxin coronatine. A major unanswered question is: "how do stomata respond to combined effect of biotic and abiotic stresses?" We found that coronatine can re-open dark-closed stomata three hours post-incubation with purified coronatine or the coronatine producing *Pst* DC3000. Same trend did not hold for the coronatine deficient mutants, *Pst* DC3118 and *Pst* DB29. We also have evidence that high RH (95%) reduces bacterium-triggered stomatal closure. The same effect was not observed under low RH (60%). These results suggest that guard cells prioritize their response when exposed to multiple stimuli. Understanding this process should help elucidating the effectiveness of stomatal-based defense in nature where plant experiences constant influx of external stimuli.

Structure of *Sclerotinia sclerotiorum* within and among lettuce fields in California

P. CHITRAMPALAM (1), Z. K. Atallah (1), B. Wu (2), K. V. Subbarao (1)
(1) University of California, Davis, Salinas, CA, U.S.A.; (2) Oregon State University, Madras, OR, U.S.A.
Phytopathology 100:S24

Genetic structure of *Sclerotinia sclerotiorum* among and within lettuce fields, as well as in individual lettuce plants was analyzed using 16 microsatellite markers. Isolates were collected from four fields, including 2 that were sampled twice at 3-weeks interval. Based on the lettuce drop incidence, one or two 25 × 25 m2 plots with multiple diseased plants were sampled in each field and all infected lettuce plants in were collected. In addition, 10 isolates were obtained from four randomly chosen lettuce heads. Results from PCR with SSR markers revealed high genetic variability in *S. sclerotiorum* populations both among and within lettuce fields. Although, several haplotypes were present in all four fields, their frequency varied significantly among fields. No correlation was observed between MCGs and SSR haplotypes. Furthermore, the analysis of *S. sclerotiorum* from single lettuce heads revealed high

genotypic variability, suggesting that inoculum from genetically different sclerotial sources could infect the same lettuce head establishing a mechanism for recombination. This hypothesis is currently being tested experimentally.

Fusarium avenaceum as causal agent of root rot in field peas and its control

K. CHITTEM (1), L. Porter (2), K. McPhee (3), M. Khan (3), R. S. Goswami (3)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) Prosser, WA, U.S.A.; (3) Fargo, ND, U.S.A.
Phytopathology 100:S25

Fusarium root rot is a serious disease in all field pea producing areas in the United States and has become a major constraint in the North Central region over the past years. Traditionally, *Fusarium solani* f. sp. *pisi* was considered to be the primary causal agent of this disease, but recent surveys conducted in North Dakota identified *Fusarium avenaceum* as the most prevalent pathogen associated with pea root rot in this state. The objectives of our study were to assess variation in aggressiveness of *F. avencaeum* isolates from field peas, evaluate commercial varieties for resistance to *F. avencaeum* and *F. solani* f. sp. *pisi*, and to estimate the ability of waste-lime, a by-product of the sugar industry, as a soil amendment to control this pathogen. The sand corn-meal layer method was used for studying the variation in aggressiveness, and for screening cultivars for resistance to both pathogens under greenhouse conditions. Significant variation in aggressiveness was observed among isolates and some isolates of *F. avenaceum* were more aggressive than isolates of *F. solani* f. sp. *pisi*. Twenty one cultivars of green and yellow peas were screened for the resistance against both *F. avenaceum* and *F. solani*, and only cv. Franklin was found to be partially resistant to both pathogens. Use of waste lime resulted in significant reductions in growth of both root rot organisms in laboratory tests and reduced the disease severity in inoculated greenhouse trials.

RpfC control tctE/tctD expression and mutations in the genes reduced virulence of Xanthomonas oryzae pv. oryzae KACC10859

J. CHO (1), M. Jeong (2), W. Kim (3), J. Cha (2)
(1) Chungbuk National Univ, Cheongju, SOUTH KOREA; (2) Cheongju, SOUTH KOREA; (3) Iksan, SOUTH KOREA
Phytopathology 100:S25

The significantly down regulated genes in the rpfC- mutant, which was screened by microarray analysis. The result was confirmed tctE/tctD genes that were known as a two-component signal transduction system involved in tricarboxylate transport in Salmonella typhimurium. Marker exchange mutations in tctE/tctD reduced the lesion length to about 65% of wild type's. Virulence factor of Xoo such as xylanase, cellulase, motility and polysaccharide (EPS) were also decreased in the mutants. Complementation of the mutations restored the virulence and virulence-related phenotypes. Reduction of tctE/tctD expression in the rpfC- mutant was confirmed by the transcription real-time PCR and assay of tct promoter activity which was fused to the GFP reporter. RpfC was known an important virulence regulator in Xanthomonas campestris and the closely related bacterial pathogens. These results suggest that RpfC controls tctE/tctD expression and the tctE/tctD are involved in virulence expression in the Xanthomonas oryzae pv. oryzae KACC10859.

Discovery of key transcription factor-encoding genes for pathogenicity in the plant-pathogenic fungus *Alternaria brassicicola*

Y. CHO (1)
(1) University of Hawaii At Manoa, Honolulu, HI, U.S.A.
Phytopathology 100:S25

Necrotrophic fungi are responsible for about 80% of the annual economic losses in agriculture worldwide, although they account for just 4% of fungal diversity. The necrotrophic fungus *Alternaria brassicicola* causes black spot disease of brassicaceous plants, including the oil-producing *B. napus* and model plant *Arabidopsis*. In order to identify key transcription factors (TFs) involved in pathogenesis, we have produced targeted gene disruption mutants for 160 of the 400 TF genes in *A. brassicicola*. Our bioassays on cabbage leaves, identified one pathogenicity factor with mutants that were nonpathogenic, and six strong and two weak virulence factors whose mutants showed respectively 50–90% and 20–49% reduction in disease symptoms compared to the wild type. We also discovered a unique gene whose mutants showed a 100% increase in virulence. We suspect the role of the group with decreased virulence was as positive regulators and the group with increased virulence was a negative regulator for downstream pathogenesis genes. Nine of these ten genes discovered in this study were novel virulence factors and only one gene (*PacC*) was previously identified as a pathogenicity factor in other fungal species. The newly discovered genes associated with pathogenicity or virulence will lead to an understanding of the orchestrated regulation of fungal genomes during pathogenesis. Such detailed information can be used to tailor efficient strategies for the management of necrotrophic fungi.

Application of Fungal Secretome Database to identify effector proteins in the plant pathogenic fungi

J. CHOI (1), J. Park (1), D. Kim (1), K. Jung (1), S. Kang (2), Y. Lee (1)
(1) Department of Agricultural Biotechnology, CeFP, CFGR, and CAB, Seoul National University, Seoul, SOUTH KOREA; (2) Department of Plant Pathology, The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S25

Fungi secrete proteins which have various functions to adapt various niches. Some of them in pathogens function as effectors that manipulate and/or destroy host cells. To enhance prediction accuracy for secretory proteins in fungi, we constructed Fungal Secretome Database (FSD; http://fsd.snu.ac.kr/) with an ensemble pipeline using nine prediction softwares. Accuracies of the pipeline showed 99.16% and 84.17% for 2,512 experimentally verified SPdb proteins and the 1,093 characterized fungal secretory proteins; while SignalP 3.0 presents 89.81% and 75.30%, respectively. To present all information intuitively, the FSD presents two main features: summary page and Favorite, a personalized repository implemented in Comparative Fungal Genomics Platform (http://cfgp.snu.ac.kr/). When 158 fungal/oomycete genomes were applied to the FSD pipeline, 208,883 proteins (15.21%) were predicted as secretory. The presence of putative effectors containing known host targeting signals such as RXLR or RXLX[EDQ] was investigated. Much higher frequency of RXLX[EDQ] was found in fungi compared to RXLR, suggesting that the RXLX[EDQ] may be one of fungal-specific signatures of effectors. FSD could serve as an integrated platform that supports researches on secretory proteins, especially in effector proteins, in the plant pathogenic fungi.

Generation of reactive oxygen species via *NOXa* is important in pathogenicity in *Mycosphaerella graminicola*

Y. CHOI (1), S. B. Goodwin (1)
(1) USDA-ARS, West Lafayette, IN, U.S.A.
Phytopathology 100:S25

Mycosphaerella graminicola is an important wheat pathogen causing significant economic loss. *M. graminicola* is a hemibiotroph, indicating that a biotrophic stage with nutrient uptake and a necrotrophic stage associated with a possible toxin or reactive oxygen species (ROS) are important to pathogenicity. To better understand the pathogenic mechanisms of *M. graminicola*, we employed over-expression strategies; selected target genes for over-expression were *CREA*, *AREA*, and *NOXa*, which might function as regulators in nutrient acquisition and ROS generation. Increased expressions of *CREA*, *AREA*, and *NOXa* were confirmed via q-RT PCR and subsequently used for pathogenicity testing. Among them, the *NOXa* over-expression strain, NO2, resulted in significantly increased pathogenicity. Moreover, instead of the usual filamentous growth, we observed a significant predominance of yeast-like growth in NO2, which is correlated with ROS production. Our data indicate that ROS generation via *NOXa* is important to pathogenicity as well as development in *M. graminicola*.

Gene encoding a c-type cyclin in *Mycosphaerella graminicola* is involved in melanin biosynthesis, stress response, and pathogenicity

Y. CHOI (1), S. B. Goodwin (1)
(1) USDA-ARS, West Lafayette, IN, U.S.A.
Phytopathology 100:S25

Mycosphaerella graminicola is an important wheat pathogen causing septoria tritici blotch. To date, an efficient strategy to control *M. graminicola* has not been developed. More significantly, we have a limited understanding of the molecular mechanisms of *M. graminicola* pathogenicity. In this study, we attempted to characterize an *MCC1*-encoding c-type cyclin, a homologous gene to *FCC1* in *Fusarium verticillioides*. Four independent *MCC1* knock-out mutants were generated via Agrobacterium-mediated transformation (ATMT). All of the *MCC1* mutants showed consistent multiple phenotypes. We could observe significant reductions in radial growth on PDA in all of the *MCC1* mutants. In addition, *MCC1* gene deletion mutants produced less mycelium on PDA, had increased melanin biosynthesis on agar plates, showed an increase in their stress tolerance response, and were reduced significantly in pathogenicity. These results indicate that the *MCC1* gene is involved in multiple signaling pathways including pathogenicity in *M. graminicola*.

Regulation and functional characterization of *Monilinia fructicola* polygalacturonase genes *MfPG1*, *MfPG2*, *MfPG3* and *MfPG5*

C. CHOU (1), R. M. Bostock (2), M. Lee (1)
(1) Department of Plant Pathology, National Chung Hsing University, Taichung, TAIWAN; (2) Department of Plant Pathology, UC Davis, Davis, CA, U.S.A.
Phytopathology 100:S25

Monilinia fructicola, the causal agent of peach brown rot, secretes a number of endopolygalacturonases (endo-PGs) that can degrade pectin, a major component of plant cell walls. *MfPG1* encodes an endo-PG, the expression of

which is up-regulated by the host phenol caffeic acid (CA) and down-regulated by H_2O_2. However, brown rot development in inoculated peach fruit and total PG activity in *M. fructicola* culture filtrates are inhibited by the presence of CA. CA can act as an antioxidant to lower the intracellular redox potential of fungal cells. H_2O_2, a pro-oxidant, is generated in response to infection by pathogens and its production may facilitate disease development caused by necrotrophic pathogens. The results suggest complex redox control of endo-PG expression in *M. fructicola*, and possibly differential regulation of other PG genes in response to changes in the redox environment during infection. Three additional *M. fructicola* endo-PG genes – *MfPG2*, *MfPG3* and *MfPG5* – were isolated by degenerate and inverse PCR and sequenced. Phylogenetic analysis revealed that these genes are highly related to endo-PG genes of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Potential redox-sensitive transcription factor binding sites were identified within all *MfPG* promoter regions, suggesting that their expression is also regulated by redox changes. Studies on the role of *MfPG1* in the *M. fructicola*-plant interaction and the regulation of *MfPG2*, *MfPG3*, and *MfPG5* will be presented.

Influence of row covers on muskmelon pollination in the absence of bacterial wilt

X. CIBILS STEWART (1), E. Saalau Rojas (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S26

Yield losses of >80% can occur on muskmelon due to bacterial wilt, a disease caused by *Erwinia tracheiphila*. The bacterium is transmitted by two species of cucumber beetles; disease management requires suppression of the beetles. Row covers placed over plants until 10 days after the start of bloom can control bacterial wilt, but could potentially impede pollination if the row covers blocks the access of pollinating insects. To test this possibility, we compared muskmelon yield in two row-cover treatments (row cover ends opened at anthesis, and bumble bees inserted under the row cover) with adjacent control plots (row cover removed at anthesis, and row cover removed 10 days after anthesis). Rows were 30.5 m long. Incidence of bacterial wilt was minimal during the study due to very low populations of cucumber beetles. Yield was lower in the middle of the rows than at the ends, suggesting that pollinators behavior was influenced by position in the row. Yield was higher in control than treatment plots. Delaying row cover removal until 10 days after anthesis also delayed harvest. These preliminary results suggest that, in the absence of bacterial wilt, delaying row cover removal until 10 days after anthesis can delay and even reduce muskmelon yield.

Factors influencing the production of *Maize fine streak virus* proteins in Drosophila S2 cells

F. M. CISNEROS (1)
(1) The Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S26

Maize fine streak virus (MFSV) is negative-sense RNA virus in the family Rhabdoviridae. Our goal is to determine whether the viral proteins required for synthesis of the MFSV genomic RNA can be produced in Drosophila S2 cells. We previously demonstrated that the MFSV nucleoprotein (N) and phosphoprotein (P) could be expressed from pMT/V5-His-Topo vector in S2 cells for at least 4 days. In contrast, expression of the MFSV replicase protein (L) was not detected in S2 cells under the same conditions. This could be due to the frequent changes in its sequence observed when the plasmid was propagated in E. coli. To avoid this problem, we are testing the expression of the L protein using linear DNA fragments (LDF) instead of circular plasmids. We have generated a LDF containing only eukaryotic regulatory elements for expression of foreign genes in Drosophila S2 cells flanking the MFSV-L gene by means of PCR. Preliminary results suggest that the MFSV-L protein can be produced in S2 cells, and experiments are underway to optimize the expression of the L protein. In addition, expression of the T7 RNA polymerase needed for synthesis of the MFSV genomic RNA in vitro was tested. Our results indicated that the T7 polymerase was expressed in S2 cells for at least 4 days when fused to a nuclear localization signal peptide (NLS), but did not accumulate when lacking the NLS. These results are important in order to optimize the conditions for the production of an infectious full-length clone of MFSV in S2 cells.

Presence of airborne inoculum of *Mycosphaerella graminicola* and occurrence of sexual reproduction during the growing season in Belgium

A. Clinckemaillie (1), G. Dedeurwaerder (1), M. Duvivier (2), J. Moreau (2), A. LEGREVE (1)
(1) Université catholique de Louvain, Louvain-la-Neuve, BELGIUM; (2) Centre wallon de Recherches agronomiques, Gembloux, BELGIUM
Phytopathology 100:S26

The impact of the sexual cycle of *Mycosphaerella graminicola* on the evolution of the Belgian population was studied using two approaches. First, the airborne inoculum of the fungus produced by sexual reproduction was collected using Burkard volumetric spore traps sited in two fields. The quantification of *M. graminicola* by qPCR revealed the occasional presence of airborne inoculum from January till December: peak release occurred not only in the autumn and winter but also in the spring and summer. The comparable temporal profile of airborne inoculum in the two fields, located 18 km apart, indicated a comparable infection pressure due to this inoculum. Second, the development of the teleomorph stage was monitored during the growing season by collecting wheat and assessing the release of ascospores from their leaves. Ascospores were obtained from the beginning of June 2009 on F4 and F1 leaves, and then sporadically on F1 to F3 leaves until July. The airborne inoculum detected in fields in the summer could therefore be explained by the presence of mature pseudothecia produced in field. The sexual cycle thus also plays a role not only by giving rise to secondary infection, but also by allowing the fungus to complete many sexual cycles within a season. Control strategies using fungicides have thus to be adjusted to limit the evolution of fungicide resistance in *M. graminicola* populations due to recombination occurring between the different treatments during the season.

Relationship of fungal and bacterial seed microflora to soybean seed vigor

K. A. COCHRAN (1), J. Rupe (1), A. Steger (1), R. Holland (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S26

Seedborne pathogens can greatly reduce soybean seed performance. To determine the effect of seed infection on soybean seed germination and vigor seven soybean cultivars with a range of reactions to *Phomopsis longicolla* and *Cercospora kikuchii* were planted at Kibler, AR, in 2008 and 2009. Plots were treated or untreated with azoxystrobin. In 2008, half of each plot was promptly harvested and the other half was harvested three weeks later. In 2009, harvest was delayed due to rain. Vigor was accessed by standard germination (SG), accelerated aging (AA), the Seed Vigor Imaging System (SVIS), and plating of seed. In 2008, *P. longicolla*, and *C. kikuchii* was negatively correlated with SVIS vigor for the first harvest. No relationship was found between SG or AA and recovery of pathogens for the first or second harvest. In 2009, there was a negative correlation between the incidence of *P. longicolla*, and *Bacillus subtilis* with SG and AA. Seed infection by *P. longicolla* and *C. kikuchii* were significantly affected by cultivar. In 2008, cultivar and fungicide affected *C. kikuchii* recovery, and in 2009, they affected *P. longicolla* recovery. Germination and vigor were higher in the second harvest in 2008 and vigor was higher in 2009 in response to the foliar fungicide. Under low disease pressure (2008), soybean SG and AA were not related to seed infection, but SVIS was. Under higher disease pressure (2009), SG and AA were negatively correlated with increases in some pathogens.

Climate change and potato late blight suppression

J. P. Comstock (1), D. W. Wolfe (1), L. Joseph (1), A. T. Degaetano (1), W. E. FRY (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S26

We used a mechanistic computer simulation model of potato late blight in combination with historical weather and predictions of future weather to assess possible effects of climate change on potato late blight and on the amount of fungicide required to suppress the disease. We used historical and projected weather data for Rochester NY. In general, weather was more favorable for disease and there was greater variance in the disease severity and amount of fungicide needed to suppress disease in 1977-2008 than in 1947-1966. The simulation model requires hourly temperature, relative humidity and precipitation data. These variables were available from state-of-the-art global climate model (GISS, GFDL, UKMO, NCAR and MIROC) projections. In all cases the variables were statistically downscaled to achieve the higher spatial and temporal resolution needed for the late blight simulation. Climate change effects were estimated using a low emissions scenario and a high emissions scenario. In both scenarios, average temperatures increased. In the low emissions scenario, disease severity and fungicide necessary to suppress disease in 2040-2067 remain similar to these values in 1977-2008. In the high emissions scenario, fungicide needed to suppress disease in 2040-2065 increased by 20–25% when compared to that required in 1977-2008.

Candidate gene silencing in *Glycine max* using amiRNA to identify soybean cyst nematode resistance gene(s) at the *Rhg1* locus

D. E. COOK (1), S. Melito (2), T. Hughes (1), A. MacGuidwin (1), A. F. Bent (1)
(1) University of Wisconsin, Madison, WI, U.S.A.; (2) University of Sassari, Sassari, ITALY
Phytopathology 100:S26

Soybean cyst nematode (SCN), *Heterodera glycines*, is the most economically damaging soybean pathogen in the U.S. Once SCN is present in a field it

cannot be eradicated, and SCN is now endemic in major soybean-producing counties across the Midwest. Two major loci, *Rhg1* and *Rhg4*, have been identified in multiple genetic mapping studies, accounting for a significant portion of SCN disease resistance. It is important to identify the genes underlying resistance at these loci to elucidate their mechanism of action and to possibly broaden the scope of SCN resistance used today. We have employed *Agrobacterium rhizogenes* mediated soybean transformation, a unique amiRNA gene silencing vector, and a nematode demographic assay to evaluate candidate resistance genes. Our recent efforts have focused on identifying the *Rhg1* locus gene(s) because it is the resistance source in a majority of commercial cultivars. Recent research indicates that the proposed LRR-kinase at *Rhg1* is not responsible for controlling a significant portion of the resistance seen in PI88788-derived sources. Results from testing other candidate SCN resistance genes at the *Rhg1* locus will be presented.

A bacterial pathogen uses distinct type III secretion systems to alternate between host kingdoms

V. R. CORREA (1), D. R. Majerczak (2), E. Ammar (3), M. Merighi (2), R. C. Pratt (1), M. G. Redinbaugh (4), D. L. Coplin (2), S. A. Hogenhout (5)
(1) Dept. of Horticulture and Crop Science, OARDC/The Ohio State University, Wooster, OH, U.S.A.; (2) Dept. of Plant Pathology, The Ohio State University, Columbus, OH, U.S.A.; (3) Dept. of Entomology, OARDC/The Ohio State University, Wooster, OH, U.S.A.; (4) USDA, ARS Corn and Soybean Research Unit / Dept. of Plant Pathology, OARDC / The Ohio State University, Wooster, OH, U.S.A.; (5) Dept. of Disease and Stress Biology / The John Innes Centre, Colney, Norwich, UNITED KINGDOM
Phytopathology 100:S27

Gram-negative bacterial pathogens of eukaryotes often secrete proteins directly into host cells via a needle-like protein channel called a 'type III secretion system' (T3SS). Bacteria that are adapted to either animal or plant hosts use phylogenetically distinct T3SSs for secreting proteins. Here, we report that *Pantoea stewartii* subsp. *stewartii* (*Pnss*), the causative agent of Stewart's wilt in maize, carries phylogenetically distinct T3SSs that enable it to invade its insect and plant hosts. In addition to a Hrp-type T3SS, known to be essential for maize pathogenesis, *Pnss* has a second T3SS (PSI-2) that is required for persistence in its flea beetle vector, *Chaetocnema pulicaria*. PSI-2 belongs to the Inv-Mxi-Spa T3SS family typically found in animal pathogens. Mutagenesis of the PSI-2 *psaN* gene, which encodes an ATPase essential for building the structural components of T3SS and secretion of T3SS effectors, greatly reduced both the persistence of *Pnss* in flea beetle guts and its transmission to maize. Ectopic expression of the *psaN* gene complemented these phenotypes. In addition, the relative expression level of the PSI-2 *psaN* gene was higher in insects compared to maize tissues. When mechanically inoculated, the *Pnss psaN* mutant was fully virulent on sweet maize, indicating that PSI-2 is not required for plant pathogenicity. Our findings demonstrate that the two T3SS in *Pnss* play different roles in the life cycle of this bacterium as it alternates between insects and plants.

Exploring attraction of *C. elegans* to the Brown Garden Snail, *Helix aspersa*

J. A. Cotton (1), K. R. SANCHEZ (1), E. P. Caswell-Chen (1)
(1) University of California, Davis, CA, U.S.A.
Phytopathology 100:S27

We isolated nematodes and bacteria associated with the Brown Garden Snail and assessed the attractiveness of snail exudates (slime) and bacterial associates to well-fed *C. elegans* (Ce N2) and food-deprived Ce. T-shaped patterns in PDMS (polydimethylsiloxane) were placed on agar to serve as bioassay arenas. Stimuli were placed at one end of the "T" cross-arm and nematodes at the base of the vertical leg. Nematodes moved up the vertical leg of the "T" and could move in two directions at the cross-arm - toward a stimulus at the end of the cross arm or toward the blank control chamber opposite. The stimuli were snail mucus, bacteria from snail mucus (*P. putida*, *S. kitahiroshimense*, *E. coli*), and *Lysobacter enzymogenes* (C3). Ce was attracted to mucus and to all of the bacteria included as stimuli, with the attraction to bacteria being greater than to mucus. Well-fed Ce were attracted to snail stimuli - 94% choosing C3 vs the control chamber, 98% choosing all snail bacteria, and 78% choosing snail mucus. Food-deprived Ce were attracted - 98% choosing snail bacteria vs the control chamber, and 67% choosing snail mucus. *L. enzymogenes* was attractive and induced vivipary in Ce. These results demonstrate possible roles of attraction in the association of Ce with their snail hosts.

Status of dodine resistance and possibilities for renewed use against *Venturia inaequalis* populations in the Northeastern U.S.

K. COX (1), S. Villani (1), G. Jacon (2)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY, U.S.A.; (2) Groupe Agriphar, Ougree, BELGIUM
Phytopathology 100:S27

The development of site-specific fungicide resistance in *Venturia inaequalis* populations in the Northeastern U.S. have left apple producers with few options for managing apple scab. Producers now rely on calendar-based applications of multi-site protectant fungicides to manage the disease. Decline in the frequency of dodine resistant isolates within a population was previously demonstrated for two orchards in the region. To investigate the prevalence of declining dodine resistance, we surveyed 93 commercial, 6 baseline, and 18 research apple orchards from 2007–2009 for sensitivity to dodine using microscopy-aided relative growth assays. Less than 27% of the orchards surveyed had *V. inaequalis* populations with practical resistance to dodine. Field trials were also conduced in an orchard formerly resistant to dodine, but with a current population displaying reduced sensitivity. Dodine programs were as effective or improved over standard programs of protectant and site-specific fungicides for managing apple scab. Following applications of dodine in the orchard, dodine sensitivity, expressed as population mean percent relative growth, increased from 36.0 ± 3.0% in 2008 to 51.4 ± 6.0% in 2009. Although, the majority of the orchard populations in the survey were composed of sensitive isolates or those with reduced sensitivity to dodine, it remains to be seen whether dodine resistant *V. inaequalis* populations will re-emerge following renewed use.

Real-time PCR detection of the Southern corn rust pathogen *Puccinia polysora*

J. CROUCH (1), L. J. Szabo (1)
(1) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A.
Phytopathology 100:S27

Southern rust, caused by *Puccinia polysora*, is an increasingly problematic disease of corn (*Zea mays*) in the U.S. The fungus has been present in North America since at least 1897, with epidemics occurring episodically throughout the 20th century in Africa, China, Central and South America. Although primarily a foliar disease, *P. polysora* may also infect sheaths and husk leaves, causing severe and early senescence. Stem lodging may also occur as an indirect result of photosynthate loss, and yield reductions may be considerable. Southern rust may be distinguished from common corn rust caused by *Puccinia sorghi* through expert examination of pustule color, pustule location, and spore morphology, but the differences between the two diseases and the causal organisms may be subtle or even impossible to detect, especially in early stages of disease development. Therefore, to reliably differentiate between these two pathogens, a real-time PCR assay based on the nuclear ribosomal internal transcribed spacer region has been developed for *P. polysora* and *P. sorghi*. This assay will be useful for monitoring and evaluating the distribution and incidence of southern corn rust in the U.S.

Sequenced restriction-associated DNA (RAD) markers for SNP discovery in the genus *Colletotrichum*

J. CROUCH (1), P. Oudemans (2), J. Polashock (3)
(1) USDA ARS, Cereal Disease Lab, St. Paul, MN, U.S.A.; (2) Rutgers University, Chatsworth, NJ, U.S.A.; (3) USDA ARS, Chatsworth, NJ, U.S.A.
Phytopathology 100:S27

Colletotrichum species are among the most widespread and important plant pathogens. Efforts are ongoing to better understand genetic variation within this genus, but resources for the high-throughput discovery and development of SNPs and other markers are currently very limited. In this study, we set out to determine whether Illumina-sequenced RAD (restriction-associated DNA) tags could be used to efficiently identify SNPs from *Colletotrichum* species, with the *C. graminicola* genome sequence assembly serving as a reference. 28,699 RAD tags were sequenced from a sample of 59 *Colletotrichum* isolated from grasses and cranberry, including *C. graminicola*, *C. cereale*, *C. acutatum* and *C. gloeosporioides*. 39% of these sequences were mapped to the *C. graminicola* genome, from which 4537 unique loci were identified. ~50% of the mapped loci possessed two or more alleles (between 2-19 alleles/locus, avg 3.9), with 1-6 SNPs present in each 49-bp sequence. For marker development, the greatest number of polymorphic loci was identified from grass-derived isolates of *C. graminicola* and its closest relatives (*C. navitas*, *C. nicholsonii*), while far fewer alleles were observed from the wider comparisons with the isolates from cranberry (*C. acutatum* and *C. gloeosporioides*). Cluster analysis of the binary coded allelic dataset shows a correspondence between the SNPs and known relationships previously inferred through nucleotide sequence analysis and RFLP markers.

SM3: An intracellular paralog of the proteinaceous elicitor SM1 from *Trichoderma virens*

F. K. CRUTCHER (1), L. J. Dangott (1), C. M. Kenerley (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S27

The biocontrol agent, *Trichoderma virens*, has the ability to protect plants from pathogens by eliciting plant defense responses, involvement in mycoparasitism, or secreting antagonistic secondary metabolites. SM1, an

elicitor of induced systemic resistance, was found to have three paralogs within the *T. virens* genome. The paralog *sm3* is highly expressed in the presence of plants and during mycoparasitism of *Rhizoctonia solani*. Comparison of culture filtrate and tissue extracts by SDS-Page and Western blots indicated that SM3, unlike SM1, is intracellular, indicating that SM3 may interact with other organisms only during direct contact with *T. virens*. SM3 was purified using anion exchange and gel filtration chromatography and sequenced for comparison to SM1. To determine the localization of the protein within cells SM3 was tagged with red fluorescent protein (RFP). The potential role of SM3 was assessed by testing the purified protein for its ability to induce six defense related genes in maize in comparison with induction by SM1. Gene deletion and over-expression mutants were generated and compared with the wild type for induction of resistance in maize against *Colletotrichum graminicola*. Understanding the roles of elicitor protein families can greatly increase our understanding of plant-microbe interactions and will give us new approaches to controlling plant diseases.

The role of calcium and other minerals on biofilm formation and adhesion force in *Xylella fastidiosa* cells

L. CRUZ (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S28

Xylella fastidiosa (XF) is known to infect a wide range of plant species, but its mechanism of pathogenicity remains unclear. Plant susceptibility has been mainly associated with water deficits from xylem vessel blockage caused by plant-derived tyloses or gums and the formation of bacterial biofilm. However, some of the symptoms are not fully explained by water stress. Based on the similarity between symptoms of XF infection and plant nutritional imbalances, we hypothesize that symptoms result from the capacity of the bacteria to uptake minerals from the plant during pathogenesis. Preliminary data suggests a positive correlation between increases in the concentrations of calcium and iron and the formation of biofilm *in vitro*, while a negative effect was found for copper and zinc. The addition of different concentrations of chelators to PD2 complete media was used to further corroborate these results. The specific Ca chelator ethylene glycol tetraacetic acid (EGTA) produced the highest reduction in biofilm formation. The specific Fe chelator deferoxamine (DFO) also produced a negative effect on biofilm formation. The adhesion force of the cells under high concentrations of Ca and EGTA was quantified using microfluidic chambers. The addition of Ca to PD2 significantly increased the adhesion force of XF, while EGTA significantly decreased adhesion to the substrate. These results are evidence of a role of Ca in adhesion and biofilm formation of XF *in vitro*.

Evaluation of fluensulfone for root knot nematode on tobacco

A. CSINOS (1), J. Whitehead (2), L. L. Hickman (1), S. S. LaHue (1)
(1) University of Georgia, Tifton, GA, U.S.A.; (2) Makhteshim Agan of North America, Oxford, MS, U.S.A.
Phytopathology 100:S28

Root knot nematodes are becoming an increasing problem on commercial tobacco and management is exasperated with short supplies of nematicides, high cost and lack of choice of materials. Makhteshim's MCW-2 (fluensulfone) was evaluated on flue-cured tobacco for management of Meloidogyne arenaria. 1, 2, 3 and 4 kg ai/ha of fluensulfone were applied to the soil bed as separate treatments in a 30 cm band using a CO2 pressurized back pack sprayer, and the material rototilled into the soil to a depth of 15 cm. Aldicarb was applied to the bed at the rate of 3.36 kg/ha and rototilled into the bed. Fenamiphos (3.36 kg/ha) was applied as described for MCW-2. The trial was a RCB design with single row plots 11m long, 1.1m wide replicated 6 times. Soil was a sand loam, with a history of M. arenaria on peanuts. All rates of MCW-2 had vigor ratings equal to aldicarb, except 4 kg/ha rate which was higher. Root gall ratings at harvest and larval numbers for MCW-2 at 3 and 4 kg/ha were lower than aldicarb treated plots. All treatments except fenamiphos had yields higher than the non-treated. Yields of MCW-2 were not different from the aldicarb standard.

Impact of bispyribac-sodium on annual bluegrass control and brown patch severity in tall fescue

M. A. CUTULLE (1), B. J. Horvath (2), J. Derr (3), A. Nichols (3), D. McCall (4)
(1) Virginia Tech, Ellicott City, MD, U.S.A.; (2) University of Tennessee, Knoxville, TN, U.S.A.; (3) Virginia Tech, Virginia Beach, VA, U.S.A.; (4) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 100:S28

Tall Fescue is one of the most commonly utilized turfgrasses for home lawns and other lower maintenance turf areas in the United States. *Rhizoctonia* infects tall fescue stands during hot humid conditions when tall fescue is under summer stress. The subsequent disease, brown patch is aesthetically unpleasing and can thin out the turfgrass stand leading to the germination and encroachment of winter annual weeds such as annual bluegrass. A potential postemergence herbicide for control of annual bluegrass in tall fescue is bispyribac-sodium. However, preliminary reports indicate that applications of bispyribac-sodium on tall fescue have increased its susceptibility to brown patch, thus promoting the sequential increase of summer disease and fall weed encroachment. Bispyrabac-sodium was applied at rates of 12 and 6 g ai ha-1 either April 22 and two weeks after or May 22 and two weeks after in 2009. Applying the low rate of bispyribac-sodium on May 22 resulted in greater than 60% brown patch in June, which was more than any other treatment. More brown patch lesions were also recorded for later applications of the high herbicide rate when compared to other treatment combinations. Overall, later herbicide applications increased disease severity in July and August regardless of herbicide rate. Early applications of the high herbicide rate resulted in less annual bluegrass cover when compared to all other treatment combinations with no objectionable phytotoxicity to tall fescue.

Interactions between lesion nematodes and *Pythium ultimum* on maize seedlings

M. P. DA SILVA (1), G. Munkvold (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S28

Lesion nematodes (*Pratylenchus penetrans*) are known to interact with root rot pathogens on a variety of host plants. The objectives of this research were to measure the effects of *P. penetrans* infestation on seedling disease symptoms caused by the fungal pathogen *Pythium ultimum*, and assess the impacts of seed treatments on this interaction. Growth-chamber experiments were conducted with 150-ml pots that were filled with an autoclaved sand-soil mixture combined with fungal inoculum (colonized corn meal/sand mixture). A suspension of 4000 *P. penetrans* (adults and juveniles) was added to the pots at the time of planting. A factorial experimental design was used including 8 seed treatments × 4 pathogen combinations × 4 replicates. Experiments were harvested 30 days after planting. Shoot lengths, fresh and dry shoot and root weights, and visual root health scores were determined. Roots were scanned and image analysis conducted with WinRhizo software; root length, volume, tips, branching, discoloration and diameter class distribution were determined. The results indicate significant effects on root health with interactions between fungal pathogens and nematodes. WinRhizo color analyses indicate significant interactions between seed treatment and nematodes affecting root health, length and volume. Root diameter class distribution was significantly affected by nematode - fungus interactions that resulted in a lower proportion of smaller diameter roots.

Identification of soybean lines resistant to Frog eye leaf spot at ultra-low plant density

R. R. DABALA (1), W. D. Clark (1), S. K. Kantartzi (1)
(1) Southern Illinois University, Carbondale, IL, U.S.A.
Phytopathology 100:S28

Frog eye leaf spot caused by *Cercospora sojina* is an emerging problem in Southern Illinois favored by hot and humid weather. The aim of this study was to screen soybean (*Glycine max* (L.) Merr.) advanced breeding lines and to identify the potential resistant ones. In total, 24 advanced breeding lines along with seven checks including one resistant-'Davis' and one susceptible-'Blackhawk' were tested through honeycomb selection, under the ultra-low density of 1.2 plants/m2 in Carbondale IL in 2009. Data showed that there were significant differences in disease severity among lines, indicating genetic variability for FLS resistance. All the 24 lines subsequently tested for the possible presence of previously reported QTLs using microsatellite markers located on LG J. An integrated analysis of phenotypic and molecular data may be useful in developing soybean cultivars with broad resistance to FLS and adapted to Southern Illinois area.

Mating between *Aspergillus flavus* cryptic species I and II

K. E. DAMANN (1), C. DeRobertis (1), R. Sweany (1)
(1) Department of Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 100:S28

Aspergillus flavus was recently shown by others to be a sexually reproducing Ascomycete and given the name *Petromyces flavus* for its teleomorphic stage. Geiser, Pitt & Taylor (1998) reported phylogenetic divergence in a collection of Australian isolates of *A. flavus* which they named cryptic species I and II. We have determined the ability of 28 of their isolates to mate *in vitro*. Pairings of *Mat 1-1* and *Mat 1-2* isolates within and between cryptic species revealed successful matings in both circumstances. This is interesting because, as originally reported, some cryptic species II isolates produce aflatoxins G1 and G2, a trait which taxonomists have recently banished from the *A. flavus* repertoire. The inheritance of aflatoxins B1, B2, G1, G2, cyclopiazonic acid, sclerotial size, and the segregation of SSR haplotypes are reported.

Comparing ectomycorrhizal colonization on transgenic, hybrid, and wildtype *Castanea dentata*

K. M. D'AMICO (1), T. R. Horton (1), C. A. Maynard (1), W. A. Powell (1)
(1) SUNY-ESF, Syracuse, NY, U.S.A.
Phytopathology 100:S29

The introduction of the fungal pathogen *Cryphonectria parasitica* (causal agent of the chestnut blight) devastated populations of American chestnut (*Castanea dentata*) across the eastern United States, effectively removing this heritage tree species from the landscape. Efforts to develop an American chestnut tree that is more resistant to blight include transformation with genes to enhance the plant's defense response. After a transgene has been introduced into a plant, it is necessary to assess the non-target impacts that the gene product might have on associated microbial populations. This study compares the ectomycorrhizal colonization of American chestnut transformed with a gene for oxalate oxidase to wildtype [American], Chinese chestnut (*Castanea mollissima*), American x Chinese chestnut (*C. dentata* x *C. mollissima*), Red Oak (*Quercus rubra*), and American Beech (*Fagus grandifolia*). Trees were grown in soils collected in the field in a soil bioassay to bait for mycorrhizal fungi. Mycorrhizal root tips were quantified and fungi were identified from mycorrhizal root tips using RFLP and sequence analysis of the fungal ITS region. The results of this study will increase our understanding of ectomycorrhizal colonization in these Fagaceae species and inform the deregulation process as part of a larger effort to restore American chestnut to its natural range.

***Physalis peruviana* natural reservoir for *Phytophthora infestans* in the field**

G. DANIES (1), A. M. Vargas (1), C. A. Antolínez (1), G. Peña (1), A. J. Bernal (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA
Phytopathology 100:S29

Phytophthora infestans is an hemibiotrophic plant pathogen that attacks a great variety of crops belonging to the family Solanaceae, including *Physalis peruviana* (cape gooseberry). Today Colombia is the leading producer of cape gooseberry in the world. The aim of this study was to contribute to the knowledge of the infection process of *P. peruviana* by *P. infestans* following the development of the disease through an histological analysis and quantitatively determining the expression of the biotrophic and necrotrophic markers *ipiO* and *npp1* respectively using qRT-PCR. Furthermore, we compared the effect of infected cape gooseberries and potatoes as sources of inoculum for cape gooseberries or potatoes in the field and in laboratory conditions. Through the histological analysis it was possible to evidence sporangia and zoospore germination. Sporulation and macroscopic symptoms were observed sporadically. The genes *ipiO* and *npp1* showed unexpected patterns of expression. Cape gooseberry plants ecotype Colombia showed to be resistant while potatoes were susceptible to the *P. infestans* inoculum circulating during the summer of 2009 in the northeast of the United States. Our results suggest that different cape gooseberry ecotypes, might play an important role in determining whether the plant is a host or a non-host. Infected cape gooseberries may serve as inoculum for cape gooseberries and potatoes, making this new host a possible source for resistant genes.

Acholeplasmavirus P1 from *Acholeplasma palmae*, an ancestral relative of plant pathogenic phytoplasmas

R. E. DAVIS (1), R. Jomantiene (2), Y. Zhao (1), I. Lee (1), E. L. Dally (1), J. Shao (1)
(1) USDA-Agricultural Research Service, Beltsville, MD, U.S.A.; (2) Nature Research Center, Vilnius, LITHUANIA
Phytopathology 100:S29

Phytoplasmas are cell wall-less prokaryotes that descended from an acholeplasma-like ancestor and exist as transkingdom parasites of plant phloem and phloem-feeding insects. Survey sequencing of the genome of *Acholeplasma palmae*, a cell wall-less prokaryote isolated by others from rotting tissues of a lethal yellowing phytoplasma-infected coconut tree, revealed the presence of virus-related sequences. Cloning and results from nucleotide sequence analysis indicated that *A. palmae* contained an extrachromosomal, circular DNA molecule encoding putative proteins that shared amino acid sequence similarities with proteins encoded by the enveloped, double-stranded circular DNA acholeplasmavirus L2 from *A. laidlawii*. Although the *A. palmae* virus shared similarities with acholeplasmavirus L2, the *A. palmae* virus, termed acholeplasmavirus P1, was distinct from L2. Results from comparative genomics revealed no homologous potential protein coding regions (open reading frames, ORFs) in partially or completely sequenced phytoplasma genomes. The findings are consistent with the concept that acholeplasmaviruses L2 and P1 invaded *Acholeplasma* spp. after evolutionary divergence of acholeplasmas from phytoplasmas.

Soil detection of crown and root rot of tomato caused by *Fusarium* in Sonora and Baja California (Mexico) using soil phytopathometry

M. de Cara (1), D. Palmero (2), M. Vazquez-Mundo (3), F. Camacho (1), J. TELLO MARQUINA (1)
(1) Univ of Almería, Almería, SPAIN; (2) Technical Univ of Madrid, Madrid, SPAIN; (3) Almería, SPAIN
Phytopathology 100:S29

In 2008, crown and root rotten and dead tomato plants widely appeared in several tomato fields in the states of Sonora and Baja California (Mexico). In order to find the causal agent, 13 rhizosphere soil samples from three of these fields were analyzed using the technique called "soil phytopathometry"[a]. This technique consists on planting 10 germinated seeds of tomato (cv San Pedro, susceptible to soil-borne pathogens) in a mixture of each rhizosphere sample and sterile vermiculite (1:6 w/w) contained in 1-L plastic pots. Three pots were used per sample. The plants were maintained for 60 days in a growth chamber at 23 to 26°C with a 16-h photoperiod. Thirty days after sowing, plants showed first symptoms, raising *F. oxysporum* f. sp. *radicis-lycopersici*: affected plants got wilted and exhibited crown and root rot. All samples presented diseased plants, and *Fusarium oxysporum* was the unique pathogen isolated from the rotten tissue when analyzed on malt extract agar. Pathogenicity of eight isolates on tomato was confirmed, and no pathogenicity was showed when these isolates were inoculated on sweet-pepper and eggplant. To our knowledge this is the first report of *F. oxysporum* f. sp. *radicis-lycopersici* in Sonora and Baja California. The usefulness of the technique was previously evaluated for *F. oxysporum* f. sp. *melonis* (race 1) and Melon Necrotic Spot Virus (MNSV) and its vector *Olpidium bornovanus*. [a]Geomicrobiology Journal, Volume 23, Issue 5 June 2006, pages 319-322

Influence of *Xylella fastidiosa* on mineral content of infected host plants

L. DE LA FUENTE (1), L. C. Cruz (1), J. K. Parker (1), P. A. Cobine (1)
(1) Auburn University, Auburn University, AL, U.S.A.
Phytopathology 100:S29

The bacterium *Xylella fastidiosa* (XF) infects several agronomically important crops. The infection process involves the blockage of xylem vessels responsible for the passage of water, but published work shows that water deficit in grapes does not produce the same symptoms as XF infection. Based on the similarities between mineral deficiency and XF infection symptoms, we are studying the effects of the XF infection process on the mineral content of host plants. A model system using greenhouse-cultivated tobacco (*Nicotiana tabacum* cv. SR1) was established. Leaves of XF-inoculated and buffer-inoculated control plants were collected before inoculation and periodically over a 6–10 weeks period after infection. The mineral content of the leaves was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) to look at the total mineral content of the leaf as well as the distribution throughout the leaf. For the latter, small (1 cm^2) sections were excised from the leaves and analyzed individually. Data was reconstructed following the leaf topography and plotted in contour maps. Preliminary results indicate distribution of minerals in "hot spots" in infected plants, while uninfected plants had an even distribution of the mineral content. These studies are being complemented with in vitro experiments on the influence of minerals on biofilm formation and attachment of XF. Our preliminary data in both the host and the pathogen will be discussed.

Developing a taxonomic identification system based on microsatellites of *Phytophthora* species

J. M. DEL CASTILLO (1), A. J. Bernal (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA
Phytopathology 100:S29

Phytophthora spp. is the most important genus of Oomycetes plant pathogens. Actually there are 80 described species, and most of these are primary invaders of plant tissues, and they are the causal agent of diseases in a wide range of crops and natural plants. In order to develop control strategies against *Phytophthora* spp., it is important to know the biology, mechanisms and evolutionary processes of this important pathogen. The aim of this study was to propose and validate a low cost identification system for *Phytophthora* species based on a set of polymorphic microsatellite (SSRs) markers. For this, 30 isolates from *P. infestans*, *P. andina*, *P. sojae*, *P. cryptogea*, *P. nicotianae*, *P. capsici* and *P. cinnamomi* were obtained, and 14 SSRs, potentially transferable markers between these species were chosen. Amplification conditions, including annealing temperature were standardized for several markers. All of them were assayed on high-resolution agarose, and some on polyacriyamide, and they specifically amplified in all species, showing different alleles depending on the species. Also RFLP analysis of COX region were performed, giving more tools to create an identification code to diagnose and monitor this plant pathogen.

First detection of *Phakopsora pachyrhizi* on Jicama in the United States and on Florida beggarweed in Alabama

M. A. DELANEY (1), E. Sikora (2)
(1) Auburn University Extension, Auburn, AL, U.S.A.; (2) Auburn University, Auburn, AL, U.S.A,
Phytopathology 100:S30

Soybean Rust, caused by *Phakopsora pachyrhizi*, was detected on Jicama (*Pachyrihius erosus*) for the first time in the United States in November, 2009. The pathogen was also detected on Florida beggarweed (*Desmodium tortuosum*) for the first time in Alabama *Phakopsora pachyrhizi* was observed on a potted Jicama plant grown outdoors in a residential area, as well as in a demonstration plot at Auburn University. Symptoms appeared on the upper leaf surface as chlorotic isolated areas near the leaf edge on leaves in the lower plant canopy. Symptoms on the lower leaf surface exhibited brown lesions and produced volcano-shaped pustules with urediniospores that were characteristic of *Phakopsora* sp. Pustules and uredinospores were pale tan in color. Leaves expressing disease symptoms of were analyzed using an Envirologix monoclonal antibody test kit at the Auburn Plant Diagnostic Laboratory. Symptomatic plant tissue was also sent to the USDA National Identification Services Laboratory in Beltsville, MD for further confirmation. The fungal structures present were confirmed to be *Phakopsora* sp. The samples and forwarded to the USDA National Plant Germplasm and Biotechnology Laboratory for DNA testing and confirmed as *P. pachyrhizi*. Symptomatic tissue obtained from Florida beggarweed collected in Headland, Alabama was submitted for identification in the same manner. To our knowledge this is the first report of the disease on Jicama in the United States and on Florida beggarweed in Alabama.

Effects of soybean cyst nematode infestation and resistance on *Fusarium* root rot on soybeans

M. M. DIAZ ARIAS (1), G. Tylka (1), L. Leandro (1), G. Munkvold (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S30

Fusarium species are ubiquitous in soil and cause important soybean diseases such as damping-off and root rot. At least 12 different *Fusarium* species have been reported from soybean roots but their relative aggressiveness as root pathogens is unknown. In some cases, root rots can be exacerbated by other pathogens such as the soybean cyst nematode (SCN). To determine whether SCN infestation enhances Fusarium root rot in soybean, greenhouse and field trials were conducted using varieties that differ in genetic resistance to SCN. Field plots were established in two Iowa locations (Story Co. and Hancock Co.). Soil and plant samples were collected to test for soil SCN populations and Fusarium root rot severity. In the field, the relationship between SCN resistance and root rot was not consistent. However, some SCN-susceptible cultivars such as 92M91 (Pioneer) had high root rot severity, and some SCN resistant cultivars such as L2620RX (Latham) had lower disease severity. For the Story location, regression analysis showed a poor relationship between root rot severity and yield, but a positive relationship among nematode population (0-3600 eggs/100cc soil) and disease severity. For the Hancock location, percent root rot severity explained 42.1% of the variation in yield, but there was a poor relationship between nematode populations and disease severity. Selected isolates from eight different *Fusarium* species are being used to test the interaction between SCN and *Fusarium* in greenhouse conditions.

PCR detection of aflatoxin producing strains of *Aspergillus* spp. from corn and red flour beetle

S. DOBHAL (1), D. Blazheva (2), M. Arif (1), P. Garrido (1), F. M. Ochoa-Corona (1), G. Opit (1), C. Garzon (1)
(1) Oklahoma State University, Department of Entomology and Plant Pathology, Stillwater, OK, U.S.A.; (2) University of Food Technologies, Department of Organic Chemistry and Microbiology, Plovdiv, BULGARIA
Phytopathology 100:S30

Aflatoxins are mycotoxins produced by some species of *Aspergillus* that contaminate food and feed. This study describes the development of two specific and sensitive primer pairs for PCR detection of aflatoxin-producing *Aspergillus* spp. in contaminated corn grain and red flour beetles (*Tribolium castaneum*), which have serious implications in food and agricultural biosecurity. Primers were designed using the web interface software Primer3, mFOLD and BLASTn with validated thermodynamic parameters. The primers amplify 142 bp of the aflB-aflR intergenic spacer (aflatoxin Q) and 162 bp of the conserved β-tubulin gene (*Aspergillus*-specific). The two PCR assays can detect down to 10^{-8} ng/µl of template DNA. To detect contamination of corn seeds and *T. castaneum* with *Aspergillus* spp., DNA was extracted from both, corn seeds and beetles. PCR products of the expected sizes were amplified, purified, and confirmed by direct sequencing. These two PCR assays allow the rapid detection of *Aspergillus* spp., and discriminates aflatoxin-producing and nonproducing species or strains, and would assist decision making and assessment about stored grain contamination in conjunction with toxin detection procedures.

Comparative analyses of the 'Candidatus Liberibacter' species reductive genome features

H. DODDAPANENI (1), H. Lin (2), Y. Duan (3), V. Lou (4), C. Chen (4), C. Vahling (3), Z. Lijuan (5), E. L. Civerolo (2)
(1) Carver Center for Genomics, Department of Biology, University of Iowa, Iowa City, IA, U.S.A.; (2) USDA, ARS, SJVARC, Parlier, CA, U.S.A.; (3) USDA, ARS, USHRL, Fort Pierce, FL, U.S.A.; (4) Guangxi Citrus Research Institute, Guilin, Guangxi, China, Parlier, CA, U.S.A.; (5) University of Florida, Indian River Research and Education Center, Fort Pierce, FL, U.S.A.
Phytopathology 100:S30

'Candidatus Liberibacter' species are gram-negative α-proteobacteria that are associated with some destructive plant diseases such as citrus Huanglongbing and potato 'zebra chip'. These bacteria are transmitted by psyllids and are classified into four species. Using whole genome amplification and 454 pyrosequencing, we have sequenced the genomes of 'Ca. Liberibacter asiaticus' (Las) and 'Ca. Liberibacter solanacearum' (Lso). A total of 1,136 and 1,126 CDS were predicted in Las and Lso, respectively. Comparative genomics and metabolic pathway analyses have revealed some details, such as the presence of reductive oxidative phosphorylation, the reduction or complete absence of metabolic enzymes and secretion systems in these genomes. There are 867 conserved proteins, of which, 531 proteins shared ≥70% similarity and may represent the core genome of the 'Ca. Liberibacter' species. The remaining 336 proteins showed greater diversification and a significant portion of those are associated with membrane and transport functions, and that may help define their speciation. Similarly, Lso and Las genomes differ by > 25% quantitatively among six functional COG categories. Five of these categories are related to external interactions that are associated with a pathogenic lifestyle. The reduced metabolic capabilities, which reflect their fastidious nature, along with the presence of pseudogenes suggest ongoing genome decay in this group of bacteria.

Response of late blight resistant tomato lines to Florida genotypes of *Phytophthora infestans*

R. S. DONAHOO (1)
(1) University of Florida, Immokalee, FL, U.S.A.
Phytopathology 100:S30

Late blight of potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) is caused by the Stramenopile *Phytophthora infestans*. Late blight is common in south Florida during the winter months, where both crops are produced, and environmental conditions are extraordinarily favorable for disease development. Over the past five years, a shift in *P. infestans* populations recovered from tomato has been observed. In an attempt to assess late blight resistance to *P. infestans* isolates collected in Florida, seed from 13 cultivars were obtained from the Asian Vegetable Research and Development Center. Six different late blight resistance genes (*Ph*+, *Ph*-1. *Ph*-2, *Ph*-3, *Ph*-4, and *Ph*-6) have been introgressed into these 'differential lines'. Using a detached leaf assay, resistance to five Florida *P. infestans* genotypes are being compared to that of the susceptible tomato cultivar 'FL-47'. The results of the detached leaf assays and the implications of P. infestans race structure in Florida will be presented.

Effect of poultry litter on *Heterodera glycines* reproduction

P. DONALD (1), P. Allen (2), K. Sistani (3), D. Tyler (4), H. Tewolde (5)
(1) USDA ARS, Jackson, TN, U.S.A.; (2) Knoxville, TN, U.S.A.; (3) Bowling Green, KY, U.S.A.; (4) Jackson, TN, U.S.A.; (5) Mississippi State, MS, U.S.A.
Phytopathology 100:S30

Soybean cyst nematode (SCN), *Heterodera glycines*, management in soybean production relies on use of incompletely resistant cultivars to reduce SCN reproduction and associated potential risk of yield loss. A poultry litter study was initiated to change soil biological composition and potentially reduce SCN reproduction. Our objective was to use Normalized Difference Vegetation Index (NDVI), soybean yield, plant height, leaf area index (LAI), and SCN egg population density to quantify the impact of poultry litter application on SCN reproduction and plant response. Data were collected for three years as part of a field study with two rates of poultry litter applied annually in the spring compared with conventional fertilizer application. Plots receiving chicken litter had significantly higher yield in 2008 ($P = 0.002$) and 2009 ($P = 0.03$) than plots fertilized with conventional fertilizer. The 2007 growing season was especially dry and no treatment differences were significant. NDVI and LAI were good predictors of plant height and soybean yield for all years. Post-harvest SCN egg population density was inversely correlated with yield ($r = –0.47$, $P = 0.003$) during 2007, but was positively correlated with yield in 2008 ($r = 0.61$, $P < 0.0001$) and 2009 ($r = 0.30$, $P = 0.06$). Significant response of SCN egg population density to treatment may

have been masked by a strong anisotropic gradient present in the field. Geostatistical analysis is being included to account for this.

Effect of light on germination, germ tube growth, and infection of daylily by *Puccinia hemerocallidis* and of geranium by *Puccinia pelargonii-zonalis*

W. DONG (1)
(1) University of Georgia, Griffin, GA, U.S.A.
Phytopathology 100:S31

The presence of rusts of daylily and geranium caused *Puccinia hemerocallidis* and *P. pelargonii-zonalis* can result in reduced value of these ornamental crops. Controlled environment experiments were conducted to determine the effects of light on urediniospore germination, germ tube growth, and infection of the two pathogens on detached leaves and plants. Exposure of non-hydrated or hydrated spores of *P. hemerocallidis* and *P. pelargonii-zonalis* to cool white fluorescent light (600µmol $s^{-1}m^{-2}$) or to sunlight (1200-1600 µmol $s^{-1}m^{-2}$) for 4 h significantly reduced germination on detached leaves. Germ tube growth of *P. hemerocallidis* was reduced by exposure to fluorescent light and sunlight for 4 h on detached leaves of daylily. Germ tube growth of hydrated spore of *P. pelargonii-zonalis* was reduced by a 4 h sunlight treatment, however, exposure of both hydrated and non-hydrated spores to fluorescent light or exposure non-hydrated spores to sunlight did not reduce the germ tube length on detached leaves of geranium. The infection rate of *P. hemerocallidis* on detached leaves of daylily decreased after 4 h treatment with fluorescent light. Exposure of inoculated plants to sunlight for >2 h decreased the infection rates of *P. hemerocallidis* and *P. pelargonii-zonalis* with a larger effect observed on plants inoculated with hydrated compared to dry urediniospores.

Effects of environment and cultivar on charcoal rot development in soybeans

M. DOUBLEDEE (1), J. Rupe (1), C. Rothrock (1), S. Bajwa (1), A. Steger (1), R. Holland (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S31

Charcoal rot, caused by *Macrophomina phaseolina*, is a soilborne disease associated with hot, dry weather, however the above ground disease symptoms are difficult to distinguish from those of drought. To separate the affects of disease from drought, four soybean cultivars, DT97-4290, DPL 4546, R01-581F, and LS980358 were grown in microplots with soil that was either infested or non-infested with *M. phaseolina*. Half of the plots were kept well watered and the other half were allowed to water stress. Stomatal conductance, canopy temperature, and spectral reflectance were measured periodically though out the season. Yield, root/stem disease severity, plant height stem discoloration and *M. phaseolina* colonization were determined at harvest. On some dates, water stressed plants and infested plants had lower stomatal conductance and higher canopy temperatures (based on infared radiation expressed as Crop Water Stress Index) than well watered or non-infested plants. At flowering in 2008, plants in infested, non-irrigated plants had lower stomatal conductance than those in infested irrigated or non-infested irrigated or non-irrigated plants. Spectral reflectance, disease assessment, and yield data will also be presented. These results suggest that infection with *M. phaseolina* may limit the water uptake in the plant, before the onset of visible symptoms.

Distribution of *Arabis mosaic virus* on vineyards in northern provinces of Iran

H. Doustseddigh (1), F. RAKHSHANDEHROO (1), M. Shamsbakhsh (2)
(1) Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Plant Pathology Department, College of Agriculture, Tarbiat Modarres University, Tehran, IRAN
Phytopathology 100:S31

Arabis mosaic virus (ArMV) is a *Nepovirus* with a very wide natural host range. Because of a extra decrease of vineyard products for 5 recently years in 2 Iran provinces (Eastern and Western Azarbaijan) and observation of doubtful symptoms to virus infection, a survey was conducted to determine the incidence of *Arabis mosaic virus* in mentioned vineyards and also Tehran province during the years 2008 to 2009. DAS-ELISA test was done with ArMV specific polyclonal antiserum. According to the ELISA test results a total number of 203 out of 453 tested leaf samples (44.8%) were infected with ArMV with the infection rate of 61.3%, 12.5% and 9.5% for Eastern, Western Azarbaijan and Tehran provinces respectively. Symptoms related to ArMV infection were recorded as spotting (concentric rings), mottling, mosaic and yellowing in surveid regions. Total RNA isolation performed with LiCl from mechanically inoculated cucumber plants according to the published protocols. Using specific primers for the coat protein of ArMV, extracted RNA samples were detected by RT-PCR method. A DNA fragment of 519bp was amplified for serological positive ArMV samples. The amplified fragments of four isolates sequenced and then aligned with the corresponding data available for other ArMV isolates in NCBI. Phylogenetic analysis revealed that of all Iranian tested isolates together with some NCBI isolates were categorized in one cluster while all other ArMV isolates from NCBI were categorized in a separate cluster.

Evaluation of Thiazosulfene nematicide drip applications to manage root-knot nematode (*Meloidogyne* spp.) on yellow squash

J. G. DRIVER (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S31

Southern root-knot nematodes (*Meloidogyne incognita*) cause severe losses to vegetable growers in the southeast U.S. Root-knot nematode damage results in plant stunting, wilting, chlorosis and yield loss. Thiazosulfene is a new non-fumigant nematicide with potential for less crop phytotoxicity. Thiazosulfene pre-plant drip applications were made in combination with thiazosulfene post-plant drip applications at various rates, and were compared to oxamyl (Vydate) and 1,3-dichloropropene (Telone II). Yellow squash (*Cucurbita pepo*) were grown on LDPE white plastic mulch and root-knot galling and yield was compared. The effect of thiazosulfene rates and application sequence was not significant among the treated plots. However, the untreated control had the highest root galling index and was significantly different than all other treatments except thiazosulfene applied at 4.17 l/ha ($P < 0.05$). The combination of a pre and post applications had no effect on root galling. Plants tested with oxamyl had similar gall severity to thiazosulfene and 1,3-dichloropropene was particularly effective at reducing root-knot galling ($P < 0.05$). Thiazosulfene applied at 6.25 l/ha and oxamyl applied as a pre and post plant treatment had similar results to 1,3-dichloropropene. Plants grown with drip applied nematicides had intermediate vigor, with the exception of plants grown with thiazosulfene at 4.17 l/ha rate that showed poor vigor.

A broad host range tailocin from *Burkholderia*

I. DUARTE (1), G. Wang (1), J. J. Gill (1), R. F. Young (1), J. J. LiPuma (2), C. F. Gonzalez (1)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) University of Michigan Medical School, Ann Harbor, MI, U.S.A.
Phytopathology 100:S31

The *Burkholderia cepacia* complex (Bcc) consists of at least 17 phenotypically similar but genotypically distinct species of non-fermenting, Gram-negative bacteria that are found in a diverse set of niches. Members of the Bcc can be involved in beneficial or pathogenic interactions with plants. They are also considered opportunistic pathogens, especially for persons with cystic fibrosis. Essentially all Bcc clinical isolates demonstrate broad-spectrum antibiotic resistance *in vitro*. Combination antibiotic therapy typically results in poor clearance of Bcc from infected individuals. There is a substantial need to develop new strategies for antimicrobial therapy against these pathogens. The use of phage-tail-like high molecular weight bacteriocins, or "tailocins", as a potential anti-bacterial agent against Bcc is under investigation. We have identified a tailocin, designated Bcep0425, which exhibits broad host range biocidal activity against members of the Bcc and other bacterial genera. We have conducted genetic analysis of the tailocin encoding genes and determined a high degree of similarity to defective phages identified in sequenced *Burkholderia* genomes, except for the tail fiber components which appear to be novel. Deletion analysis of tailocin structural genes will be used to further characterize Bcep0425.

Pathogenic variation of *Pectobacterium carotovorum* isolates and the effects of relative humidity on the severity of bacterial stem rot in potato

J. K. Dung (1), B. K. SCHROEDER (1), D. A. Johnson (1)
(1) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S31

Bacterial stem rot (BSR) of potato, usually caused by *Pectobacterium carotovorum* (*Pc*), causes lesions and soft rot of aerial stems. The effects of isolate and relative humidity (RH) were examined by syringe-inoculating stems of "Umatilla Russet" potato plants with *Pc* cells (10^6 CFU) suspended in sterile distilled H_2O (sdH_2O). Two *Pc* isolates obtained from BSR lesions (O207.1 and V104.1), two *Pc* isolates collected from soft rot tubers (Ec101 and 1.1.2009) and a sdH_2O control were used. Plants were subjected to 90–100% (high) RH or 20–30% (low) RH for 24 hr post-inoculation (p.i.) and placed in a greenhouse for evaluation. Areas under the lesion progress curve (AULPC) were calculated from lesion length measurements taken at 2, 4 and 8 days p.i. Stems and progeny tubers were sampled and plated onto CVP to detect pectolytic bacteria. Significant ($P \leq 0.0006$) effects of isolate, RH and isolate × RH interaction on AULPC values were observed. AULPC values were significantly greater with isolates O207.1, V104.1 and 1.1.2009 than

with the sdH_2O control at both RH treatments and were significantly greater at high RH than low RH. Isolate Ec101was not significantly different from the sdH_2O control at low RH but was significantly greater at high RH. Pectolytic bacteria were recovered from ≥70% of inoculated stems but were not isolated from progeny tubers. These results indicate that isolates of *Pc* may vary in their capacity to cause BSR lesions and RH may affect symptom severity.

Management of Fusarium wilt of watermelon with fungicides

D. S. EGEL (1), K. Everts (2)
(1) Purdue University, Vincennes, IN, U.S.A.; (2) University of Maryland College Park, Salisbury, MD, U.S.A.
Phytopathology 100:S32

Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *niveum* (FON) is a limiting factor in watermelon production across the eastern U.S. An increase in FW has occurred with the phase out of methyl bromide as a soil fumigant, the spread of race 2 of FON, and the increasing production of triploid cultivars. Thus, the search for chemical management options has increased. The U.S. national program IR-4 initiated trials of soil-applied chemicals to manage FW. In 2008 in Indiana, Delaware and Maryland, fungicides were applied once at transplanting. In Delaware, acibenzolar-S-methyl, thiophanate-methyl, prothioconazole and ipconazole significantly reduced wilt at 2 1/2 weeks. In Maryland, all treatments except azoxystrobin reduced wilt incidence at 4 and 5 weeks. In Indiana, none of the treatments decreased wilt as compared with the control, but propiconazole, and metconazole increased vine vigor at 37 and 46 days. In 2009 at two sites, acibenzolar-S-methyl, prothioconazole and thiophanate-methyl were applied alone and in combination, through trickle irrigation immediately after transplanting and 2 and 4 weeks later. In Maryland, prothioconazole used alone, or with either acibenzolar-S-methyl and/or thiophanate-methyl reduced wilt at 31 and 45 days. In Indiana, prothioconazole reduced wilt when used alone or in combination with thiophanate-methyl whereas prothioconazole used in combination with acibenzolar-S-methyl did not reduce wilt.

Black leg of basil caused by *Plectosporium tabacinum* is reported in the United States

D. S. Egel (1), G. S. RUHL (2), S. Hoke (1), B. Dicklow (3), R. Wick (3)
(1) Purdue University, Vincennes, IN, U.S.A.; (2) Purdue University, West Lafayette, IN, U.S.A.; (3) University of Massachusetts, Amherst, MA, U.S.A.
Phytopathology 100:S32

Dark, irregular, black stem lesions of sweet basil (*Ocimum basilicum* 'Genovese') were observed in a hydroponic greenhouse in Indiana in 2007. Diseased plant samples were sent to diagnostic clinics at Purdue University and the University of Massachusetts. A fungus identified as *Plectosporium tabacinum* was cultured from the basil stems. Inoculations were performed on rooted basil plants in each of eight, 125-ml Erlenmeyer flasks. Four flasks were filled with 100 ml of deionized water as negative controls and four were filled with a 1×10^6 CFU/ml water suspension of *P. tabacinum*. After 24 h incubation on a laboratory bench at 23C, the solutions in all flasks were discarded and each flask and root system was rinsed three times with deionized water and incubated an additional 7 days in deionized water. Dark brown-to-black stem lesions similar to those described above developed at the base of the hypocotyl at the water interface and extended to a mean of 22 mm above the water interface on inoculated plants. Control plants remained symptomless. *P. tabacinum* was recovered from symptomatic tissue of inoculated plants to complete Koch's postulates. These data indicate that *P. tabacinum* was the causal agent of the symptoms observed on the hydroponic basil. To our knowledge, this is the first report of *P. tabacinum* causing 'black leg' and reduced growth on basil in the United States and the first report in the world on hydroponic basil.

Genetic diversity of Anastomosis Group 3 of *Rhizoctonia solani* isolates from potato in Iran by PCR-RFLP

F. EGHBALIAN (1), H. Zamanizadeh (1), B. Morid (2), S. Hajmansoor (3)
(1) Department of Plant Pathology, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Department of Plant Protection, Takestan Branch, Islamic Azad University, Tehran, IRAN; (3) Science and Research Branch, Islamic Azad University, Tehran, IRAN
Phytopathology 100:S32

Rhizoctonia solani is one of the most important agents of potato losses in Iran that causes stem canker and black scurf of tuber. Anastomosis Group 3 (AG-3) is known as the major cause of the diseases. Recent researches have shown that AG-3 isolates from potato and tobacco are distinct in genetic diversity. In order to study genetic diversity in AG-3 population from potato, thirty one isolates of *Rhizoctonia solani* were collected from different regions of Iran. The anastomosis groups were determined by observation of hyphal interactions. Thirty isolates were identified as AG-3 and one isolate as AG-4. Molecular analysis was done based on PCR-RFLP by specific primers for rDNA IGS1 region. The size of the PCR products were 680 bp in AG-3 isolates and 650 bp in AG-4 isolate. To evaluate genetic diversity in AG-3 group, thirteen restriction enzymes (seven 4-cuttrer and six 6-cutter) were used while *BamH* I was just able to show the diversity among isolates; consequently, AG-3 isolates were divided into two different groups. No correlation was observed between genetic diversity and geographical regions.

Annular rings for enhancing photographs of perineal patterns of root-knot nematodes

J. D. EISENBACK (1)
(1) Virginia Tech, Blacksburg, VA, U.S.A.
Phytopathology 100:S32

Morphology of the perineal pattern of female root-knot nematodes remains useful for routine identification of *Meloidogyne* species. A new technique that uses an annular ring in the stage condenser produces images with increased resolution and enhanced surface morphology that look like images produced with a scanning electron microscope. Annular rings are most often mounted in the stage condenser of a light microscope for phase microscopy. They are necessary for phase and have been shown to increase resolution. This increase in resolution occurs from the coherent illumination (light waves that vibrate with constant phase relationships) produced by the annular aperture that is absent in the normal brightfield condenser. The initial picture appears similar to a photographic negative but is inverted into a positive with image processing software and a computer. This new technique of illumination may increase the value of the observation of perineal patterns for identification of *Meloidogyne* species by increasing resolution and enhancing surface morphology.

Time spray strategies for Septoria leaf blotch disease progress on winter wheat: The use of forecasting model

M. El Jarroudi (1), F. Giraud (2), P. Delfosse (3), L. Hoffmann (3), H. Maraite (4), B. TYCHON (5)
(1) Univ of Liege, Arlon, BELGIUM; (2) Martillac, FRANCE; (3) Belvaux, LUXEMBOURG; (4) Louvain-la-Neuve, BELGIUM; (5) Arlon, BELGIUM
Phytopathology 100:S32

A mechanistic model, PROCULTURE, was used over the 2003–2009 period to simulate septoria wheat leaf blotch progression in the canopy in four-replicated field experiments located in three villages (Diekirch district: Reuler; Grevenmacher district: Burmerange and Christnach), representative of the different agroclimatological zones of the Grand-Duchy of Luxembourg. This model has been developed in order to find the optimum time of fungicide spray in fields. On the basis of simulated disease progression on the upper leaves. A weekly PROCULTURE recalibration is routinely done using actual disease levels observed on site. The results indicated that the relationship between disease control by fungicides and yield loss varies from site-to-site and from season-to-season. On average, only one application of fungicide is required to control efficiently the Septoria leaf blotch disease. PROCULTURE forecasts have been shown to be correct in about 85% of all cases. The fungicide treatment determined by the simulation model over 2003–2009 period resulted in a better return on investment (80%) than the other single treatments tested and as important as the double fungicide application (GS31 and GS 59) for Everlange, Christnach and Burmerange. At Reuler, between 2003 and 2009, treatments based on the Septoria risk simulation model were recommended only once, in 2007. The climatic conditions of this last site tend to favour organic farming in this region where foliar disease pressure is very weak.

Assessment of the night weather parameters and their use in forecasting model of leaf rust

M. El Jarroudi (1), F. Giraud (2), P. Delfosse (3), L. Hoffmann (3), H. Maraite (4), B. TYCHON (5)
(1) Univ of Liege, Arlon, BELGIUM; (2) Martillac, FRANCE; (3) Belvaux, LUXEMBOURG; (4) Louvain-la-Neuve, BELGIUM; (5) Arlon, BELGIUM
Phytopathology 100:S32

A stochastic model was developed to predict the wheat leaf rust (Puccinia triticina Eriks.) severity (percentage of leaf area with symptoms showing uredinia) in four-replicated field experiments located in three villages (Diekirch district: Reuler; Grevenmacher district: Burmerange and Christnach), representative of the different agroclimatological zones of Luxembourg. The model was elaborated by the analysis of the night weather and leaf rust incidence. Statistical validation using regression analysis reports a strong correlation between the number of hours with specific meteorological conditions and the percentage leaf area covered by brown rust lesions for the two upper and youngest leaves, which are mostly responsible for photosynthesis activity and assimilates production filling the grains. The development of the brown rust requires a period of at least twelve consecutive

hours with temperatures between 8 and 16°C and a relative humidity (RH) greater than 60%, with optimal values lying between 12 and 16°C and RH greater than 80%. During the 2004 to 2009 period, at four sites, the linear regression between simulated and observed values for Puccinia triticina was highly significant ($P < 0.01$) and R2 (coefficient of determination) explained 80 to 85% of the variability. Efforts are now being developed to better define thresholds for fungicide applications and to spatialize the outputs of the model over the entire Luxembourg territory.

Effect of climate change on plant-pathogen-beneficial microorganism interactions

Y. ELAD (1), O. Agra (2), H. Ben Kalifa (2), D. Rav David (2), M. Borenshtein (2)
(1) ARO, The Volcani Center, Bet Dagan, ISRAEL; (2) Volcani Center, Bet Dagan, ISRAEL
Phytopathology 100:S33

The interactions of tomato plants, yeast or bacterial potential biocontrol agents (BCAs) and either humidity-promoted diseases (late blight and gray mold) or a disease that is also active under less humid conditions (*Oidium neolycopersici* powdery mildew) were studied, in order to later model the effects of climate change on plant diseases and suggest adaptive measures. The effects of wetness duration, level of RH and temperature change on the pathogens, the survival of the BCAs and disease suppression were studied. Under high-temperature and low-RH conditions, the examined bacterium's (*Pseudomonas* sp.) survival was poorer than that of the examined yeast (*Rhodotorula* sp.). The microorganisms survived well at 10–15°C under high RH conditions. The bacterium survived better under high RH conditions than under lower RH conditions in a net house, and also survived better on leaves with powdery mildew than on symptomless leaves. The yeast was less affected by microclimatic conditions and survived for 14 days. Establishment of the two humidity-promoted diseases was lower and suppression by BCAs was better when wet conditions persisted for 8 h than at 24-h wet period, no disease control was observed. Thus, environmental conditions affect disease intensity, BCA survival and the efficacy of the introduced microorganisms. Furthermore, it is clear that such effects will occur and that adaptive measures need to be developed in order to respond to these expected changes.

Influence of time, host plant and location on diversity of aster yellows phytoplasma strains

S. Y. ELATEEK (1), M. L. Ivey (1), A. P. Michel (1), P. A. Paul (1), S. A. Miller (1)
(1) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S33

Surveys of aster leafhoppers (*Macrosteles quadrilineatus*) were conducted in the summer of 2008 and 2009 to assess the distribution of strains of aster yellows phytoplasma in two major vegetable production areas (Celeryville and Hartville) in northern Ohio. Aster leafhopper adults were collected every 2 weeks from mid-June until mid-September from red leaf, green leaf and romaine lettuce, cilantro and parsley production fields. A total of 3,330 leafhoppers were collected and tested by nested and multiplex PCR to identify phytoplasma strains. The percentage of AYP-positive aster leafhoppers peaked in August in both years and locations, and was higher in Celeryville than Hartville. Four AYP strains were identified: AY-WB (aster yellows phytoplasma subgroup 16SrI-A) and AY-BW, AY-BD2 and AY-S (16SrI-B). Some leafhoppers (13.4%) carried unknown AYP strains. AY-WB was only detected in leafhoppers collected in green leaf and romaine lettuce and parsley; 16SrI-B strains were found in leafhoppers from all crops tested. Strains AY-BW and AY-BD2 were detected in similar numbers and were predominant in 2008 in both locations, while in 2009, AY-BW was predominant in Hartville and AY-WB was most abundant in Celeryville. The distribution and/or abundance of aster yellows phytoplasma strains appear to be influenced by time, host plant and location of production fields.

Impact of gaseous ozone on postharvest fungal decays of tomato fruits

M. ELKAHKY (1), J. Bartz (2), M. El-Sheshtawi (1), S. Elafifi (1), M. Elmazaty (1)
(1) Mansoura University / Faculty of Agriculture, Mansoura, EGYPT; (2) University of Florida / IFAS, Gainesville, FL, U.S.A.
Phytopathology 100:S33

Postharvest fungal decays of tomato caused by *Alternaria alternata*, *Botrytis cinerea*, *Geotrichum candidum* and *Rhizopus stolonifer* result in significant economic losses during different stages from harvesting from farm to fork. The efficacy of ozone gas for controlling fungal postharvest fungal decays of tomato was evaluated. In the laboratory tests, Ozone 0.1 ppm in the atmosphere above inoculated PDA didn't affect radial growth, fresh weight or dry weight of the previously mentioned fungi, but, the development of aerial mycelium over the cultures appeared to be blocked. On the other hand, ozone significantly decreased the spore germination of the most tested fungi. Ozone also, decreased the density of fungal spores in the air of a storage room when it was applied for 24 hours. In fruit tests, ozone 0.15 ppm at 10°C and RH 99% prevented the development of lesions on wound-inoculated tomato fruits for 7 days. After 10 days, progressive lesions were observed but were smaller than those on control fruit. In contrast, when the treatment was applied at 22°C, lesions developed similarly to those on control fruit but sporulation was inhibited. There was no evidence of phytotoxicity associated with these ozone treatments.

Identifying resistance to *Pythium irregulare* and *Fusarium graminearum* in soybean

M. L. ELLIS (1), P. A. Paul (1), A. E. Dorrance (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S33

Pythium irregulare and *Fusarium graminearum* have emerged as important soybean seedling pathogens in Ohio. The objective of this research was to assess and characterize resistance to these two pathogens. A greenhouse assay was used to evaluate 105 soybean genotypes for potential resistance to two isolates of *P. irregulare*. Data for seed germination, total weight, root weight, and a root rot score using an ordinal scale were collected. Twenty of these genotypes were then evaluated for resistance to *F. graminearum*, using a rolled towel method. Data for seedling disease severity was collected. Based on the results for the greenhouse assay, the isolate × genotype interaction for root weight was not significant; however there was a significant difference between the two isolates and among genotypes ($P < 0.0001$). There was also a significant difference among genotypes ($P < 0.0001$) inoculated with *F. graminearum*. A number of potential candidates with high levels of resistance to both pathogens were identified.

Description of two putative new species of *Pythium* isolated from soybean and corn in Ohio

M. L. ELLIS (1), K. D. Broders (2), P. A. Paul (1), A. E. Dorrance (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.; (2) University of Guelph, Guelph, ON, CANADA
Phytopathology 100:S33

During a survey of agronomic soils from 88 locations in Ohio, a distinct morphological group of *Pythium* species, designated as group 7 (G7), was recovered from 30% of the locations and was pathogenic on both corn and soybean. The objective of this study was to use both morphological and sequence data to characterize this group. Sequence analysis of the ITS1-5.8S-ITS2 region of the ribosomal DNA for 21 isolates separated them into two distinct groups within the E1 clade of the genus *Pythium*. The first group consisted of eleven isolates that were 99% similar to *P. acrogynum* and *P. hypogynum*. The second group consisted of ten isolates that were 97% similar to *P. longandrum* and *P. longisporangium*. Therefore these two subgroups, previously designated as G7, are proposed as two new species based on morphological and sequence analysis. The frequency that these new species were isolated from agronomic production fields makes them an important component to characterize for future management of the *Pythium* complex affecting corn and soybeans in Ohio.

***Sick Plants and a Hungry World*: An online course for Master Gardeners**

S. D. ELLIS (1), P. J. Bennett (2), M. J. Boehm (1)
(1) The Ohio State University Department of Plant Pathology, Columbus, OH, U.S.A.; (2) Ohio State University Extension State Master Gardener Program, Springfield, OH, U.S.A.
Phytopathology 100:S33

What *was* the future in teaching and learning is *now* the reality. Online courses can now be found on any topic and are not just for those seeking college degrees, but also for those dedicated to lifelong learning. One group who falls under the latter category are the Master Gardeners, who are individuals from all over the United States that specialize in horticultural topics and are required to earn continuing education credits each year. With this in mind, a collaborative effort between The Department of Plant Pathology and the Ohio Master Gardener Volunteers took shape. The department took an online course currently offered asynchronously to Ohio State students and transformed it into an online course specific to Master Gardener Volunteers. The non-credit course entitled *Sick Plants and a Hungry World* is offered through the free course management system Moodle and covers topics ranging from the history of plant diseases to global issues in plant pathology. Ten modules make up the content of the course where little involvement from the instructor is needed. Students register through the Office of Continuing Education at Ohio State and have ten weeks to complete the self-paced course. Self-assessments allow students to test themselves on the material. Cost of the course is $35. Over 200 individuals from 16 states have registered for the course since its launch in March 2009.

Those completing the course receive a certificate of completion from the department.

Biological control of bacterial spot of tomato and capsicum caused by *Xanthomonas campestris* and *Pseudomonas solanacearum* by bacteriophages in the UAE

K. A. EL-TARABILY (1), F. McKenna (2)
(1) United Arab Emirates Univ, Al-Ain, UAE; (2) Natural Science Center Inc., Steele, AL, U.S.A.
Phytopathology 100:S34

Five different highly virulent and polyvalent phages were isolated from tomato and capsicum rhizosphere soil in the United Arab Emirates (UAE) using selective enrichment technique. These phages were screened for their abilities to lyse *in vitro Xanthomonas campestris* and *Pseudomonas solanacearum*, the causal agents of bacterial spot disease of tomato and capsicum in the UAE. The presence of these pathogenic bacteria in the UAE vegetable fields affects dramatically plant production and leads also to environmental pollution due to the excessive bactericide application by the commercial farmers. The phage suspension (x 10^9 pfu ml^{-1}) was prepared in especially designed mini bioreactor. These five phages were subsequently tested in the greenhouse, individually or as a mixture, for their ability to suppress the incidence of the disease. Antagonistic *Streptomyces* sp. which was found to be resistant to each individual phage was used with the mixtures of the five phages in order to improve the efficiency of the phages to reduce the incidence of the disease. The treatment which included all five phages combined with *Streptomyces* sp. was significantly superior to all other treatments in suppressing the disease severity more than the application of each phage alone or with the mixture of phages alone. Results showed that there is a potential to use a mixture of phages combined with *Streptomyces* sp. for the field management of tomato and capsicum bacterial spot disease in the UAE.

Biological control of bean broomrape (*Orobanche crenata*) and hemp broomrape (*Orobanche ramosa*) by *Fusarium* isolates

K. A. EL-TARABILY (1), M. Abouzeid (2)
(1) United Arab Emirates Univ, Al-Ain, UAE; (2) Ain Shams University, Cairo, EGYPT
Phytopathology 100:S34

Thirty-nine *Fusarium* isolates were obtained from infected bean broomrape (*Orobanche crenata*) and hemp broomrape (*Orobanche ramosa*) collected from infested fields in Egypt. The effect of *Fusarium* culture filtrates on germination of seeds of the two *Orobanche* species was tested *in vitro*. The culture filtrates of *Fusarium* species isolated from *O. crenata* were found to be more toxic to the seeds of both *Orobanche* species than those obtained from *O. ramosa*. Seeds of *O. crenata* were shown to be more resistant to *Fusarium* culture filtrates compared to those of *O. ramosa*. The highest inhibition values in germination of *Orobanche* seeds were recorded for six *Fusarium* isolates, one identified as *F. oxysporum*, one as *F. equiseti* and four as *F. compactum*. Aqueous mixtures of mycelia and conidia of all *Fusarium* isolates were directly sprayed on *O. ramosa* tubercles formed on roots of tomato plants grown in transparent plastic bags and were used also to infest soil in pots seeded with both faba bean and *O. crenata*. Two of the four *F. compactum* isolates were significantly more pathogenic against *O. crenata* and *O. ramosa*, respectively, compared to other *Fusarium* isolates tested in the pots and plastic bags. This study clearly shows the potential to use biocontrol agents originating from one *Orobanche* sp. (e.g. *O. crenata*) to control another (e.g. *O. ramosa*) as many *Fusarium* isolates originating from *O. crenata* were found to be more pathogenic to *O. ramosa* seeds than isolates originating from *O. ramosa*.

Isolates of *Phytophthora capsici* differ in their ability to cause disease on cucurbit fruits

T. B. ENZENBACHER (1), M. K. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S34

Studies were undertaken to elucidate the differences in disease response among eight types of cucurbit fruits and differences in virulence among five unique isolates of *Phytophthora capsici*. Isolates differed in mating type, mefenoxam sensitivity, and host origin. Unwounded, summer squash types and cucumbers were inoculated with a 5-mm plug of mycelia and sporangia from a 5-to 7-day-old culture and were incubated at room temperature and high relative humidity under laboratory conditions. Hard squash types were wounded with a sterile probe 3 to 5 mm below the fruit surface before being inoculated and exposed to high humidity in a greenhouse environment. Fruits were measured for lesion and pathogen sporulation diameter (cm) and evaluated for sporulation density using a visual scale. All *P. capsici* isolates used incited disease on cucurbit fruit with significant differences observed among the isolates and fruit type tested. This study suggests that multiple isolates should be utilized in future cucurbit germplasm screenings for *P. capsici* fruit resistance.

Resting spores for long-term storage of *Synchytrium solstitiale*, a candidate for biological control of yellow starthistle

F. M. ESKANDARI (1), W. L. Bruckart (1), T. L. Widmer (1)
(1) USDA ARS FDWSRU, Fort Detrick, MD, U.S.A.
Phytopathology 100:S34

An isolate of *Synchytrium solstitiale* from France has been evaluated recently 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 *S. solstitiale* resulted with mature resting spores in dried YST leaves. Leaves were surface sterilized, and resting spores 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 spores following this protocol. A test set up to measure viability and virulence of resting spores after 2, 3, or 4 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.

Effect of foliar fungicides on hail damaged corn in Wisconsin in 2009

P. D. ESKER (1), N. C. Koval (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S34

On 24 July 2009, two trials located at Lancaster, WI were impacted by 0.6 to 1.3 cm-sized hail. In both trials, plants were at the tasseling into silking growth stages. The first trial was established to study the effect of fungicide timing (V5-V6, R1, or combinations) on corn disease development (all diseases) and yield and the second to study the effect of corn hybrid ($n = 6$) and fungicide on corn anthracnose and grain yield. Both trials had four replications. Trials were sprayed on 29 July at R1. In the first trial, fungicides included pyraclostrobin, azoxystrobin+propiconazole, and propiconazole+trifloxystrobin. Only pyraclostrobin was applied in trial two. Late season disease measures included early evidence of ear rot, top dieback, lodging due to anthracnose, and common smut. Corn plants ($n = 5$) were also destructively sampled for stalk assessments (0–5 rating scale). Yield measures were moisture, test weight, and grain yield (adjusted to 15.5% moisture). In trial one, there was no evidence of a difference among treatments ($P > 0.05$) for late season diseases or yield. Grain yield ranged from 93 to 141 bushels per acre (CV = 25.1%) and the increased variability was attributed to hail. In trial two, there were differences among treatments ($P < 0.05$), but these were primarily a function of corn hybrid and no differences in yield were observed. These results suggest that hybrid selection is still the primary factor to consider for corn disease management.

Citrus greening in commercial orchards in Puerto Rico

C. ESTEVEZ DE JENSEN (1), A. Vitoreli (2), F. Roman (3)
(1) University of Puerto Rico, Juana Diaz, U.S.A.; (2) Plant Disease Clinic/University of Florida, Gainesville, FL, U.S.A.; (3) University of Puerto Rico, Mayaguez, U.S.A.
Phytopathology 100:S34

Citrus greening (CG) associated with *Candidatus Liberibacter asiaticus*, was recently identified in Puerto Rico. Symptomatic and asymptomatic leaves of sweet oranges (cv. Valencia and Washington Navel), Lime (cv. Tahiti), grapefruit and mandarin were sampled in Castañer, Juana Diaz, Yahuecas and Isabela Municipalities. Standard Polymerase Chain Reaction (PCR) was performed on DNA extractions using primers OI1 and OI2. The 16S rDNA fragments with molecular weight of 1160 bp were amplified in an agarose gel at 1.5% and corresponded to the bacterium *Candidatus Liberibacter asiaticus*. Sequencing of the PCR products from Isabela confirmed amplification of *Ca. L. asiaticus* DNA. Sweet oranges and mandarins were severely affected at Isabela where tree decline was observed. In Castañer in a 600 acre orchard two out of 25 samples were positive for the disease. In a three year old Thaiti lime orchard in Juana Diaz, symptoms developed from mottled areas and yellowed shoots to stem and limb dieback within five months in 81 out of 352 trees. Ten samples out of 25 samples were positive for *C. L. asiaticus*. A survey of citrus greening and implementation of IPM practices to prevent the spreading of the disease are needed.

Fungal and oomycete pathogens associated with crown and root diseases of strawberry in Western Australia

X. L. FANG (1), D. Phillips (2), H. Li (1), K. Sivasithamparam (3), M. Barbetti (4)
(1) University of Western Australia, Perth, AUSTRALIA; (2) Department of Agriculture and Food Western Australia, Midland, AUSTRALIA; (3) University of Western Australia, Nedlands, AUSTRALIA; (4) University of Western Australia, Crawley, AUSTRALIA
Phytopathology 100:S35

Strawberries are a high-value export crop in Western Australia (WA), constituting more than 70% of Australia's strawberry exports. Crown and root diseases pose an increasing challenge to strawberry production in WA, with more than 1 m plants p.a. dying from such diseases. Field surveys undertaken in 2008 showed that the percentage plant decline indices (%DI) across all sites ranged from 3 to 40. The mean level of plant decline across all sites rose sharply from a %DI of 13 in August to 39 in October. Based on morphological and molecular identification, the potential fungal and oomycete pathogens associated with crown and root diseases were *Fusarium oxysporum*, *Rhizoctonia* (*R. solani*, *Ceratobasidium* AG-A, AG-C, AG-I and other taxa of *Ceratobasidium*), *Cylindrocarpon destructans*, *Phoma exigua*, *Gnomonia fructicola*, *Phytophthora cactorum*, *Pythium ultimum* and *Macrophomina phaseolina*. *F. oxysporum* was most frequently isolated from crowns, at a frequency of 41%, and its incidence was strongly correlated with the severity of crown disease. *Rhizoctonia* and *C. destructans* were most frequently isolated from roots, at a frequency of 12% for each. There was a poor relationship between the incidence/severity of crown disease and root disease. This work not only demonstrates that strawberry production in WA is severely compromised by crown and root diseases, but implicates Fusarium wilt in particular as the major disease associated with the extensive plant deaths occurring in WA.

Identification and characterization of resistance to *Meloidogyne incognita* in wild species of Cucurbitaceae

T. FASKE (1)
(1) Tarleton State University, Stephenville, TX, U.S.A.
Phytopathology 100:S35

Resistance of four wild species of Cucurbitaceae to *Meloidogyne incognita* was evaluated in two greenhouse experiments. The objectives were to find new sources of resistance and determine the mechanism of resistance among resistant species. Wild species included *Cucumis melo* var. *dudaium*, *C. melo* var. *texanus*, *C. dipsaceus*, and *Cucurbita fastidissima*, which are commonly found in the southern U.S. Each entry was inoculated at the first true leaf stage with 1,000 nematodes per 500 cm^3 of soil. Only *C. melo* var. *dudaium*, *C. melo* var. *texanus* supported less ($P = 0.05$) reproduction than the susceptible control, *C. sativus* 'Straight eight'. Resistance in these species was as great as *C. metuliferus*, a resistant species. The mechanism of resistance in these species was attributed to fewer ($P = 0.05$) juveniles penetrating root tips and delayed ($P = 0.05$) maturity of juveniles into mature females, than the susceptible control. A similar mechanism of resistance was observed in *C. metuliferus*. *Cucumis melo* var. *dudaium* and *C. melo* var. *texanus* may be useful sources of resistance to *M. incognita* in honeydew and muskmelon.

Screening of organically certifiable fungicides and natural compounds to control anthracnose caused by *Colletotrichum orbiculare* in cantaloupe

M. FELICIANO-RIVERA (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S35

Control options for management of cucurbit anthracnose in organic production are poorly developed. Thus, the objective of this research was to investigate potential disease control obtained with natural, organically certifiable spray materials against *Colletotrichum orbiculare in vitro* and *in vivo*. Materials tested included: essential oils, bicarbonate salts, commercial products and chitosan. Antifungal activity was evaluated *in vitro* with 96-well plates using absorbance (450 nm) to measure mycelial growth, and/or by observing spore germination. *In vivo* experiments were performed under greenhouse conditions. Treated plants were inoculated, and disease severity was recorded using APS Assess Software 2.0. *In vitro*, bicarbonate salts ($KHCO_3$, $NaHCO_3$ and NH_4HCO_3) provided >50% inhibition at 0.25 molarity, while Bordeaux®, Kocide 2000® and SoilGard 12G® inhibited mycelial growth by >70% at concentrations ≥50 µg/ml. Horticultural lime sulfur completely inhibited spore germination at 2.5 µg/ml. Over 65% inhibition was obtained using chitosan at concentrations ≥100 µg/ml. *In vivo*, NH_4HCO_3, Serenade Max®, Bordeaux®, Kocide 2000®, SoilGard 12G®, Horticultural lime sulfur and chitosan provided >85% disease control and were statistically significant different from the non-treated plants ($P < 0.05$). None of the essential oils provided a significant reduction ($P > 0.05$) in disease. These results suggest the potential for use of these organically certifiable fungicides and natural compounds to control anthracnose in cucurbit.

A spinach BAC library for marker development, gene discovery, and functional genomics

C. FENG (1), J. C. Correll (1), B. H. Bluhm (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S35

Spinach production has increased dramatically during the past two decades in the U.S. Downy mildew remains the most economically important disease of spinach and improving genetic resistance to downy mildew is a high priority in all spinach breeding programs. A bacterial artificial chromosome (BAC) library was constructed from a spinach near-isogenic line harboring the downy mildew resistance locus RPF1. The library contains 73,728 clones with an average insert size 183 Mb, thus providing approximately 13X coverage of the 989 Mb spinach genome. An initial examination of 3535 BAC-end sequences identified gene sequences encoding conserved proteins such as kinases, endonucleases, dehydrogenases, ATPases, cellulose synthases, drought-induced proteins, low-temperature inducible proteins, permeases, germination proteins, zinc-finger proteins and other transcription factors. Over 120 BAC-end sequences contained simple sequence repeats (SSRs), including the di-[$(AT)_n$, $(AG)_n$, and $(AC)_n$], tri- [$(AAC)_n$, $(GAT)_n$, and $(CTT)_n$], tetra-[$(TTTA)_n$, $(GTTG)_n$, $(ACAT)_n$], and penta- $(TAGAC)_n$ nucleotide repeats. Di- and tri-nucleotide repeats were most prevalent. Polymorphic repeats were detected among selected cultivars, and heterozygosity of some loci was detected. This BAC library will assist with gene discovery, the development of markers linked to important traits such as disease resistance, and the integration of genetic and physical maps with genomic sequence.

The *CYP51C* gene, a novel marker for phylogenetic analysis of *Fusarium* species

D. Fernandez-Ortuno (1), E. Loza-Reyes (1), S. L. Rogers (1), B. A. FRAAIJE (1)
(1) Rothamsted Research, Harpenden, UNITED KINGDOM
Phytopathology 100:S35

The *CYP51* gene encodes sterol 14α-demethylase, a key enzyme in the pathway leading to ergosterol, phytosterol and cholesterol biosynthesis in fungi, plants and mammals, respectively, and the target of triazole fungicides. Common resistance mechanisms towards triazoles are *CYP51* mutation and over-expression. Some filamentous ascomycetes, including *Aspergillus fumigatus* and *Rhynchosporium secalis*, have two *CYP51* genes, *CYP51A* and *CYP51B*. However, a third copy, *CYP51C*, has only been identified in *Fusarium* species and appears to be unique to this genus. In this study, we investigated if the *Fusarium*-specific *CYP51C* gene can be used as an informative marker to differentiate *Fusarium* species present in cereals. Molecular and phylogenetic analyses based on 46 *CYP51C* sequences of 18 *Fusarium* species revealed sufficient sequence variability to differentiate between them. The species-dependent separation showed clear correlation between major phylogenetic lineages and abilities to synthesise particular classes of toxins. The interspecific divergence was used to design species-specific primers. The resulting PCR assays differentiated *F. asiaticum/F. vorosii*, *F. cerealis*, *F. equiseti* and *F. poae* from other members of the Fusarium head blight complex. Early detection and control of different *Fusarium* spp. is crucial to prevent toxins entering the food chain and a useful tool in disease management practices.

Bacterial communities associated to *Eucalyptus* plants infected by *Ceratocystis fimbriata*

A. FERREIRA (1), E. R. Gonzáles (2), F. D. Andreote (3), J. Azevedo (1), W. L. Araújo (4)
(1) Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, BRAZIL; (2) Suzano Bahia Sul Papel e Celulose S/A, Suzano, SP, BRAZIL; (3) Embrapa Meio Ambiente, Jaguariúna, SP, BRAZIL; (4) Laboratory of Molecular Biology and Microbial Ecology, NIB, University of Mogi das Cruzes, SP, Mogi das Cruzes, SP, BRAZIL
Phytopathology 100:S35

Brazil is one of the largest producers of *Eucalyptus* in the world, and the demand for this wood has increasing in the last few years. The expansion of the cultivation area results in changes of factors influencing eucalyptus growth and incidence of some pathogens such as *Ceratocystis fimbriata*. The pathogen-plant interaction may be affected by host ecology and associated microbial communities. Therefore, we used *C. fimbriata* infected plants with four infestation symptoms to assess the effects of this infestation on rhizoplane and endophytic bacterial communities. The culturable bacterial diversity was assessed by isolation and 16S rRNA gene characterization by ARDRA and sequencing. Additionally, total bacterial diversity was assessed by the culture-independent approach DGGE. Results showed that healthy plants presented a higher bacterial density in the rhizoplane, while the endophytic community was higher in infected plants. Thirteen and eight ARDRA ribotypes were observed in the cultured bacteria isolated from

rhizoplane and endosphere of roots, respectively. The most frequent species was *Bacillus cereus*. However, only the occurrence of species *Pseudomonas fluorescens*, *P. veronii* and *Rahnella aquatilis* were correlated with less disease occurrence. The DGGE analysis showed that the pathogen infestation interferes in the composition of the assessed bacterial communities. Principal Components Analysis (PCA) on the basis of DGGE band patterns separated samples in the four stages of disease infection. The results obtained in the present work provide information about the pathogenic-associated microorganisms and show important features related to *C. fimbriata* infection.

ITS sequence analysis of Brazilian *Stenocarpella macrospora* and *Stenocarpella maydis* isolates

A. FESSEHAIE (1), G. Munkvold (2), E. Alves (3)
(1) Seed Science Center, Iowa State University, Ames, IA, U.S.A.; (2) Dept. of Plant Pathology, Iowa State University, Ames, IA, U.S.A.; (3) Pioneer Sementes Ltda., Rodovia GO 210 km 04, BRAZIL
Phytopathology 100:S36

Stenocarpella macrospora and *S. maydis* cause ear rot, stalk rot, and leaf diseases in maize. These diseases can cause considerable yield losses in maize in warm, humid environments. The objective of this work is to assess the occurrence and distribution of *Stenocarpella* species in maize producing regions of Brazil and evaluate variability among isolates based on geographic origin. *Stenocarpella* species were isolated from maize kernels and leaf tissues obtained from representative locations, with two independent isolations made from each kernel and leaf sample. Pure cultures were identified based on morphological characteristics and the analyses of nucleotide sequence of the rDNA ITS regions. *Stenocarpella* species were isolated from 100% (12 of 12) and 37.5% (5 of 16) of the kernel and leaf samples, respectively. *S. macrospora* occurred in nine of the twelve kernel samples as a sole contaminant. Two samples were infested by both species and only one sample was infested by *S. maydis* alone. From maize leaf samples, only *S. macrospora* was recovered. Phylogenetic analyses based on the rDNA ITS sequence data revealed that *S. macrospora* isolates formed a homogenous cluster regardless of geographic origin. *S. maydis* isolates grouped in a separate distinct cluster. *S. macrospora* was more prevalent as a pathogen of maize in Brazil. *S. maydis* occurred only in samples from southern Brazil.

Real-time PCR to measure head smut infection in maize seedlings

A. FESSEHAIE (1), G. Munkvold (2)
(1) Seed Science Center, Iowa State University, Ames, IA, U.S.A.; (2) Dept. of Plant Pathology, Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S36

Head smut caused by *Sporisorium holci-sorghi* can be a devastating disease on every continent where maize is grown. The disease can cause severe yield loss by replacing the reproductive parts of the plant with fungal sori. Evaluating control methods or genetic resistance for maize head smut is difficult because of the need for field trials that are slow, costly, and highly variable. The main purpose of this study was to develop a real-time PCR assay to evaluate maize seedlings for *S. holci-sorghi* infection that occurs during germination and emergence. We report a novel real-time PCR assay for the detection and quantification of *S. holci-sorghi* in maize seedlings. Specificity of the forward primer used in this study was reported previously. Sensitivity of the assay was determined using serial dilutions of genomic DNA extracted from pure teliospores, maize leaf tissue spiked with spores and naturally infected maize plant tissue. The detection limit of the assay for purified DNA from teliospores was 250 fg per 25 µl PCR reaction mixture. PCR detected target DNA extracted from tissues spiked with teliospores and from naturally infected maize tissue at minimum quantities of 2.5 pg and 25 pg, respectively. The new real-time PCR assay is sensitive and simple; it could be useful for more rapid evaluation of fungicide seed treatments or genetic resistance for management of *S. holci-sorghi* in maize.

Leaf blotch disease complex in Norwegian wheat

A. FICKE (1), O. Elen (1), U. Abrahamsen (1), G. Brodal (1)
(1) Bioforsk Norwegian Inst of Agric & Env Res, Aas, NORWAY
Phytopathology 100:S36

The leaf blotch disease complex (LBD) frequently reduces yield of wheat in Norway. In visual assessments field symptoms can be difficult to attribute definitively to specific causal agents, and may be caused by any or all of the following three pathogens: *Stagonospora nodorum* (teleomorph *Phaeosphaeria nodorum*) causing Stagonospora Nodorum or glume blotch (SNB), *Septoria tritici* (teleomorph: *Mycosphaerella graminicola*) causing Septoria tritici or speckled leaf blotch (STB), and *Drechslera tritici-repentis* (teleomorph *Pyrenophora tritici-repentis* causing tan spot (DTR). There is no broad resistance to all three pathogens in commercially relevant wheat varieties. We analyzed 9 years of historical data on severity of LBD in the field and 36 years of historical data on post-harvest SNB infection of wheat kernels. Overall, correlation between leaf severity and seed severity over years was low (r = 0.5). However, during the last 4 years correlation between SNB seed infection and severity of LBD increased (r = .825). LBD severity varied significantly with geographic location and increased exponentially on the last 3 leaves between BBCH stage 70 and the last assessment at BBCH stage 89. An improved understanding of environmental and host developmental factors as they affect each member of the LBD complex in the field will be essential to screening for quantitative and durable resistance to LBD.

AvrGf1 from *Xanthomonas citri* subsp. *citri* strain A^{w} targets the chloroplast in grapefruit leaves

J. FIGUEIREDO (1), N. Potnis (1), P. Romer (2), T. Lahaye (2), J. Jones (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Martinsried, GERMANY
Phytopathology 100:S36

A related strain of *Xanthomonas citri* subsp. *citri* strain A that causes Asiatic citrus canker, *X. citri* subsp. *citri* strain A^{w} (Xcc-A^{w}) isolated in Florida, is pathogenic to Key lime but not grapefruit or orange. Infiltration of Xcc-A^{w} in grapefruit leaves triggers a hypersensitive response (HR). This defense response results from the recognition of a type III effector, AvrGf1, by an unknown "resistance" gene in grapefruit. Analysis of the AvrGf1 amino acid sequence using neural-network based predictors PCLR, ChloroP and LOCtree to determine the subcellular localization, predicted a chloroplast transit signal at the N-terminus within the first 87 amino acids. To test this prediction, we constructed a T-DNA binary vector expressing AvrGf1 driven by the 35S constitutive promoter and C-terminally tagged GFP. Chloroplast localization was visualized in leaves six days after infiltration using confocal laser scanning microscopy. AvrGf1-GFP is visibly concentrated in stomatal guard cells in which chloroplasts are abundant. Two mutants carrying deletions at the N-terminus disrupting the putative chloroplast signal was designed, Gf1ΔN116, which 116 amino acids were deleted, abolished GFP expression in grapefruit leaves and HR induction. However, the mutant Gf1ΔN13, with 13 amino acids deleted, resulted in HR elicitation but not GFP expression. Thus, we suggest that disruption of the putative chloroplast signal, even in the few first amino acids, is essential for the subcellular localization of AvrGf1 protein.

Screening of St. Augustinegrass (*Stenotaphrum secundatum*) germplasm for brown patch and large patch resistance

N. C. FLOR (1), P. F. Harmon (1), L. E. Datnoff (2), R. N. Raid (3)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Department of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA, U.S.A.; (3) University of Florida, Belle Glade, FL, U.S.A.
Phytopathology 100:S36

St. Augustinegrass (SAG) is a warm season grass commonly used in the United States. Large patch disease, caused by *Rhizoctonia solani* AG-2-2 LP, is considered one of the most important fungal diseases of SAG. While brown patch disease, caused by other anastomosis groups of *R. solani* primarily affects cool season grasses, but does occasionally occur on warm season grasses as well. Differences in large patch resistance in SAG cultivars has been observed but never quantified. One BP and one LP isolate from St. Augustinegrass were chosen to screen twenty genotypes of SAG. Pots of SAG plants were inoculated with mycelial plugs and maintained in an incubator at 23 to 26°C. Disease progress on each genotype was assessed daily and the intrinsic rates of infection were calculated. AUDPC also was calculated for each genotype. Trials were replicated, randomized, and repeated. Means were separated after ANOVA with a Waller-Duncan k-ratio t-test. Results indicated that some genotypes were more resistant to large patch and brown patch than others, but results did not correlate between isolates for all genotypes. In general, dwarf genotypes of SAG developed more disease than standard height genotypes. Ploidy level within genotype did not significantly impact resistance. Future screening efforts should focus on large patch isolates of *R. solani*. Additional work is needed to further characterize brown patch isolates from warm season turfgrass and to determine the potential impact of this disease.

Hormetic effect of cyazofamid on the radial growth of *Pythium aphanidermatum*

F. FLORES (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S36

Hormesis is an adaptive biphasic response of an organism, in which low doses of an inhibitory agent stimulate, while higher doses suppress a determined endpoint. Scarce studies have focused on the occurrence of hormesis in plant pathogens. The hormetic effects of low doses of pesticides may be relevant to disease management if partial application results in stimulatory dosages at some spatial scales. The aim of this research was to assess the effect of small doses of the pesticide cyazofamid on the radial growth of *Pythium aphanidermatum* in vitro. Petri dishes containing solid growing media

amended with different doses of the pesticide were inoculated with a 5 mm diameter plug colonized by actively growing mycelium. Seven of the nine doses tested were at subinhibitory concentrations. Non-amended control plates were also inoculated. The radial growth of mycelial colonies was measured after 24 h incubation at 28°C. Five replicates for each treatment were performed with five repetitions over time. The dose response curve, inferred EC_{50} and the non observable adverse effect limit (NOAEL) were analyzed using a modified log-logistic model. Radial growth stimulation up to 13% occurred when *P. aphanidermatum* was exposed to concentrations between 0.07 and 0.13 ppb cyazofamid. These observations provide the groundwork for future studies confirming the occurrence of such responses in plant production systems.

Luna fungicides for the control of diseases of horticultural crops

L. FOUGHT (1), G. H. Musson (2), H. Young (2)
(1) Bayer CropScience, Fresno, CA, U.S.A.; (2) Bayer CropScience, Res Triangle Park, NC, U.S.A.
Phytopathology 100:S37

Luna fungicides are a family of horticultural fungicide products in development by Bayer CropScience. Luna products are based on the new active ingredient fluopyram. Four mixture products have also been submitted to the U.S. EPA as combinations with other active ingredients: trifloxystrobin, pyrimethanil, tebuconazole, and 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 blight, gray mold, and scab. Multiyear trial results will be presented.

Evaluation of the expression of genes associated with resistance to Aspergillus flavus colonization and aflatoxin production in different maize lines

J. C. Fountain (1), Z. Chen (2), B. Scully (1), R. C. Kemerait (3), R. L. Dewey (4), B. GUO (1)
(1) USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA, U.S.A.; (2) Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, U.S.A.; (3) Department of Plant Pathology, the University of Georgia, Tifton, GA, U.S.A.; (4) Department of Crop and Soil Sciences, the University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S37

Aflatoxins are carcinogenic toxic compounds produced by Aspergillus flavus during infection of crops including maize (Zea mays L.). Contamination of maize with aflatoxin is exacerbated by late season drought stress. Previous studies have implicated numerous resistance-associated proteins (RAPs) that may be responsible for resistance to A. flavus colonization and aflatoxin accumulation. This study examined the expression of three genes encoding RAPs, ZmPR-10 (PR-10), glyoxalase I (GLX-I), and a 14-kDa trypsin inhibitor (TI-14), in different maize genotypes under drought stressed and irrigated conditions to determine their potential utility as molecular markers for germplasm screening and evaluation utilizing quantitative real-time PCR. Results suggested that drought stress during kernel development affected gene expressions differently in different genotypes. Results were generally consistent with expectations in that RAP-coding gene expressions correlated well to known resistant traits of the examined genotypes. However, more genotypes should be studied in order to apply these genes' expression as selection markers.

Characterization of canker resistance in citrus plants created by 'somatic cybridization'

M. I. FRANCIS (1), A. Pena (1), J. W. Grosser (1), J. H. Graham (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S37

Cybrids are an asymmetric hybrid that contain the nucleus of one parent in combination with the mitochondrial and/or chloroplast genome of a second parent. Mitochondria and chloroplasts have a central regulatory role in integrating stress and/or programmed cell death signaling. The model cybrid (RL+MK) for evaluation was a hybrid between susceptible 'Rough' lemon (RL, *Citrus jambhiri*) and resistant 'Meiwa' kumquat (MK, *Fortunella crassifolia*.) Resistant MK developed a hypersensitive response (HR) with necrotic lesions and low population of *Xanthomonas citri* subsp. *citri* (*Xcc*) in detached leaf and in attached leaf assays. Early expression of genes related to programmed cell death was identified in MK. RL+MK cybrid produced an intermediate reaction and *Xcc* population response between susceptible and resistant parents at low *Xcc* inoculum (10^5 cfu/ml) and necrosis indicative of a resistant reaction at high *Xcc* inoculum (10^8 cfu/ml). To validate the inheritance of resistance from MK, 22 cybrids of 'Ruby red' grapefruit (RG) with MK were compared to both parents using detached leaf assays. RG+MK cybrids had significantly lower lesion number per inoculation site (20 to 88%), compared to the RG parent. *Xcc* populations in RG+MK cybrids varied from 7 to 2.2 log units. Thus, resistance in cybrids may be inherited at different levels depending which sets of genes contained in the mitochondrion or chloroplast genomes are transferred to RG in the cybridization process.

Mutations in the target protein of succinate-dehydrogenase inhibitors (SDHI) conferring changes in fungicide sensitivity

M. FRANK (1), A. Glaettli (1), S. Schlehuber (1), G. Stammler (1)
(1) BASF S.E., Limburgerhof, GERMANY
Phytopathology 100:S37

Fungicide resistance of fungal plant pathogens is mostly based on point mutations in the target proteins. Monitoring of the sensitivity of different target pathogens of the SDH Inhibitor Boscalid was carried out in the recent years. While in most species the sensitivity of all isolates were within the normal or baseline range, cases of resistance were found e.g. in *B. cinerea* (Stammler *et al.*, 2007), *Corynespora cassiicola* (Ishii, 2008) or *Alternaria alternata* (Avenot *et al.*, 2008). Target gene analysis of such isolates showed mutations in the subunits of the SDH B, C and D. The current status of SDHI sensitivity in the field will be reported. Moreover, point mutations identified in field isolates of various fungi are reviewed and their structural implications and effect on fungicide binding is discussed based on three-dimensional protein models.

Gene expression during appressorium formation by *Phakopsora pachyrhizi*

R. D. FREDERICK (1), C. L. Stone (1)
(1) USDA ARS FDWSRU, Fort Detrick, MD, U.S.A.
Phytopathology 100:S37

Phakopsora pachyrhizi, which causes Asian soybean rust (ASR), has spread from southern Asia and Australia to Africa and South America, and more recently to North America. At present, U.S. commercial soybean cultivars do not have any resistance to ASR. To develop novel methods for controlling this disease, it is important to understand the molecular processes that occur throughout the infection cycle. This study examined gene expression during appressorium formation, which is required for the pathogen to breach the leaf surface. An appressorium-enriched cDNA library was constructed with mRNA extracted from appressoria produced by germinating urediniospores on polystyrene plates and subtracting with mRNA extracted from urediniospores germinated on water. A total of 1152 cDNA clones were sequenced and compared to *P. pachyrhizi* germinating urediniospore ESTs, and 31 clones were found only in the appressorium-enriched cDNA library. BlastX analysis revealed sequence similarity to known proteins for 20 clones, and identified three clones as hypothetical proteins. Eight clones showed no significant similarity to protein sequences in GenBank. Genes identified in this study fell into functional categories of metabolism, cell cycle and DNA processing, protein fate, cellular transport, cellular communication and signal transduction, and cell rescue.

Alteration in lignin biosynthesis restricts growth of *Fusarium* species in brown midrib sorghum

D. L. FUNNELL-HARRIS (1), J. F. Pedersen (1), S. E. Sattler (1)
(1) USDA ARS, Lincoln, NE, U.S.A.
Phytopathology 100:S37

To improve sorghum for bioenergy and forage uses, *brown midrib6* (*bmr6*) and *bmr12* near-isogenic genotypes were developed in different sorghum backgrounds. *bmr6* and *bmr12* grain had significantly reduced colonization by members of the *Gibberella fujikuroi* species complex, compared with wild-type, as detected on two semi-selective media. *Fusarium* species were identified using sequence analysis of a portion of the translation elongation factor 1-α gene (*TEF*). The pathogens *Fusarium thapsinum*, *Fusarium proliferatum* and *Fusarium verticillioides*, *G. fujikuroi members*, were commonly recovered. Other frequently isolated *Fusarium* species likely colonize sorghum asymptomatically. Chi-square analyses showed that the ratios of *Fusarium* species colonizing *bmr12* grain were significantly different from wild-type, indicating that *bmr12* affects colonization by *Fusarium* spp. One *Fusarium incarnatum/equiseti* species complex (FIESC) genotype, commonly isolated from wild-type and *bmr6* grain, was not detected in *bmr12* grain. Phylogenetic analysis suggested that this FIESC genotype represents a previously unreported *TEF* haplotype. When peduncles of wild-type and near-isogenic *bmr* plants were inoculated with *F. thapsinum F. verticillioides*, or *Alternaria alternata*, the resulting mean lesion lengths were significantly reduced relative to wild-type in one or both *bmr* mutants. This indicates that impairing lignin biosynthesis results in reduced colonization by *Fusarium* spp. and *A. alternata*.

Soil and rhizosphere populations of fluorescent *Pseudomonas* spp. associated with field-grown plants are affected by sorghum genotype

D. L. FUNNELL-HARRIS (1), J. F. Pedersen (1), S. E. Sattler (1)
(1) USDA ARS, Lincoln, NE, U.S.A.
Phytopathology 100:S37

Sorghum [*Sorghum bicolor* (L.) Moench] is valued for bioenergy, feed and food. Potential of sorghum genotypes to support differing populations of root-

and soil-associated fluorescent *Pseudomonas* spp. or *Fusarium* spp., in two soils, was assessed. Pseudomonads and *Fusarium* spp. were assessed from roots and soil of field-grown sorghum genotypes Redlan and RTx433, along with biological control traits including hydrogen cyanide (HCN) and 2,4-diacetylphlorogluconol (*phl*) production. In dryland field conditions, RTx433 roots had greater numbers of pseudomonads than Redlan before anthesis but similar numbers 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 *phl* 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.

Density dependent latency in the grapevine powdery mildew (*Erysiphe necator*)

D. M. GADOURY (1), M. M. Moyer (2), R. C. Seem (2), W. F. Wilcox (2), L. M. Wakefield (2), P. A. Magarey (3)
(1) Cornell University, Geneva, NY, U.S.A.; (2) Cornell University NYSAES, Geneva, NY, U.S.A.; (3) Magarey Plant Pathology, Loxton, SA 5333, AUSTRALIA
Phytopathology 100:S38

Duration of the latent period in *E. necator* has been estimated in previous studies by depositing relatively large numbers or dense aggregations of conidia by various means upon relatively small areas of host tissue. In natural epidemics, inoculum is more commonly dispersed on air currents and arrives at the infection court as a single conidium or ascospore. *Vitis vinifera* 'Chardonnay' was inoculated using 5 mml droplets of a conidial suspension containing 1 to 250 germinable conidia. Under the most favorable environmental conditions and upon the most susceptible leaves, the latent period was relatively constant above 10 spores per inoculation point, but as density approach 1 spore per point the latent period increased by more than 50%. Unrealistically dense inoculation will therefore yield unrealistically short latent periods. Under field conditions, the longer latent periods interacted with ontogenic resistance of leaves, whose aging during latency further delayed sporulation. Mortality of hyphae in nascent colonies due to environmental factors or fungicide effects could also delay sporulation by reducing colony density below a critical level. Conversely, dense colonization typically observed on shoots emerging from dormant infected buds (flag shoots) could minimize the latent period. Thus there may be qualitative as well as quantitative differences between epidemics developing from ascosporic inoculum compared to flag shoots.

Broad spectrum virus resistance using oligoadenylate (OAS) system

L. C. GALVEZ (1), Z. Zhang (2), A. Mitra (1)
(1) University of Nebraska Lincoln, Lincoln, NE, U.S.A.; (2) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S38

Tobacco SR1 and *Benthamiana* plants expressing mammalian oligoadenylate system (OAS) system exhibit resistance to tobacco etch virus (TEV), cucumber mosaic virus strain D (CMV-D) and cucumber mosaic virus strain y (CMV-y). The system is composed of two enzymes, a 2,5A synthetase that produces 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) upon activation by viral dsRNA, and a 2-5A-dependent RNase L. Transformed plants individually carrying the two enzymes were crossed and the progenies were tested against TMV, TEV and CMV. Detached leaf assays of plants carrying the two enzymes showed necrotic lesion formation which is a manifestation of hypersensitive response (HR) against the virus infection. On the other hand, plants with RL construct alone showed a typical systemic infection. It was interesting that SR1 plants carrying 2,5A alone also manifested hypersensitive response against TEV. Transgenic soybean carrying the OAS system was also evaluated for resistance against soybean mosaic virus (SMV) and bean pod mosaic virus (BPMV). Transformed soybean leaf protoplasts inoculated with SMV were ELISA negative whereas protoplasts from control soybean showed normal viral replication. The OAS system has the potential to provide broad spectrum resistance against all + RNA and dsRNA viruses.

Production of antimicrobial compounds by endophytic bacteria

L. C. GALVEZ (1), A. K. Vidaver (1)
(1) University of Nebraska Lincoln, Lincoln, NE, U.S.A.
Phytopathology 100:S38

The *in vitro* inhibition assay for testing endophyte strains (Vidaver, 1972), was used to screen for production of antimicrobial compounds. The capability of endophytic bacteria to produce antimicrobial compounds is a major mechanism by which they qualify as candidates biocontrol agents. This study showed that antimicrobial compounds produced by Gram-positive endophytes inhibit the growth of several bacteria, fungi and oomycetes. Activity was detected against the taxonomically related *Clavibacter michiganensis* subsp. *nebraskensis*, a corn pathogen causing Goss' wilt and blight, as well as *Pantoea stewartii* subsp. *stewartii* (Pss) strains, fungi including *Rhizoctonia solani*, *Sclerotinia sclerotium*, *Bipolaris sorokiniana* and *Fusarium graminearium*, and an oomycete (*Phythium ultimum*). Inhibition zones were detected after two days at 28°C, and varied in size and appearance. Thus, the endophytes produced both bacteriocin(s) and one or more other antimicrobial compounds.

Uptake and exclusion of plant-expressed fluorescent proteins by the soybean cyst nematode *Heterodera glycines*

B. GAO (1), J. Bradley (1), M. Hresko (1), A. Caruano-Yzermans (1), J. Williams (1)
(1) Divergence Inc., St. Louis, MO, U.S.A.
Phytopathology 100:S38

Cyst nematodes, major pathogens of soybean, potatoes, sugar beets, and other crops, are sedentary root feeders that limit the size of ingested molecules by use of an organelle-like feeding tube believed to act as a "molecular sieve" between the nematode stylet and the surrounding syncytial plant feeding site. The feeding tube constrains the delivery of proteins and nucleic acids from the plant to the nematode. The size cut-off for molecular uptake into the soybean cyst nematode (SCN), *Heterodera glycines*, has never been determined and work on other cyst nematode species provides conflicting values between 20 and 40 kDa. We have therefore studied the uptake of variously sized fluorescent proteins expressed in transgenic roots into *H. glycines* in comparison to another sedentary endoparasite, root knot nematode (*Meloidogyne incognita*) and the migratory lesion nematode (*Pratylenchus scribneri*). We provide the first evidence for uptake of plant-expressed fluorescent proteins into the SCN intestine and describe this uptake by developmental stage. We show how the likelihood of uptake into the intestine decreases with molecular weight establishing a molecular size cut-off for ingestion. The results obtained from this study clarify and expand understanding of SCN host-plant feeding and provide guidance for the development of biotechnology-based strategies for nematode control.

Characterization of *EDS1* as a component of *Vitis* defense responses to *Erysiphe necator*

F. GAO (1), X. Shu (2), M. B. Ali (2), S. Howard (2), N. Li (2), P. Winterhagen (2), W. Qiu (2), W. Gassmann (1)
(1) University of Missouri, Columbia, MO, U.S.A.; (2) Missouri State University, Mountain Grove, MO, U.S.A.
Phytopathology 100:S38

Vitis vinifera is the most economically important deciduous fruit crop, but cultivated grapevine varieties lack adequate innate immunity to a range of diseases. To identify defense pathways in grapevine, we focus on orthologs of the central *Arabidopsis* defense regulator *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*). The family of *EDS1*-like genes is expanded in grapevine, and members of this family were previously found to be constitutively upregulated in the resistant variety 'Norton' of the North American grapevine species *Vitis aestivalis*, while they were induced by *Erysiphe necator*, the causal agent of grapevine powdery mildew (PM) in the susceptible *V. vinifera* variety 'Cabernet Sauvignon'. We determined the responsiveness of *Vitis EDS1*-like genes to PM and salicylic acid, and find that *EDS1*-like paralogs are differentially regulated in 'Cabernet Sauvignon', while two are constitutively upregulated in 'Norton'. Sequencing *Vitis EDS1*-like cDNA and genomic clones with highest sequence similarity to AtEDS1 revealed high conservation in the protein encoding sequence and some divergence of the promoter sequence in the two grapevine varieties. By complementing an *Arabidopsis eds1-1* mutant, we showed that *Vitis EDS1* from either grapevine variety is a functional ortholog of *AtEDS1*. Together, our analyses show that differential susceptibility to PM is correlated with differences in *EDS1* expression, not differences in *EDS1* function, between resistant 'Norton' and susceptible 'Cabernet Sauvignon'.

A role for WRKY proteins in the low 18:1-derived defense signaling pathway

Q. GAO (1), D. Navarre (2), A. Kachroo (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) USDA-ARS, Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S38

A reduction in oleic acid (18:1) levels simultaneously upregulates salicylic acid (SA)-mediated responses and inhibits jasmonic acid (JA)-inducible responses in plants. Plants containing low 18:1 exhibit enhanced resistance to biotrophic pathogens but are hypersusceptible to necrotrophic pathogens. Replenishing 18:1 levels restores both Sa- and JA-related defense phenotypes. We recently reported that SA and the signaling component, enhanced disease susceptibility 1 (EDS1) function redundantly in this low 18:1-induced pathway to upregulate SA-derived responses, including resistance to

biotrophs. Here, we show that WRKY proteins mediate the low 18:1-derived repression of JA signaling, and accumulation of SA. Knockout mutations in two *WRKY* genes lower SA levels in the low 18:1-containing Arabidopsis mutant, *ssi2*, but not to basal levels. The double mutant plants are not restored in SA-mediated signaling, and continue to express pathogenesis-related genes constitutively and exhibit enhanced resistance to *Pseudomonas syringae*. In contrast, both JA-inducible *PDF1.2* expression, and basal resistance to *Botrytis cinerea* are restored in these plants. Thus, two WRKY proteins positively regulate SA accumulation and negatively regulate JA-derived resistance signaling in the low 18:1-induced defense pathway.

Expression and homology modeling of Dihydroorotate dehydrogenase from the phytopathogenic Oomycete *Phytophthora infestans*
M. F. GARAVITO (1), L. Garcia (1), G. L. Lozano (1), A. J. Bernal (1), B. H. Zimmerman (1), S. Restrepo (1)
(1) Univ De Los Andes, Bogota, COLOMBIA
Phytopathology 100:S39

The oomycete *Phytophthora infestans* (Mont.) de Bary, the causal agent of the tomato and potato late blight, causes tremendous crop and economic losses worldwide. In Colombia this pathogen is currently a devastating risk for highlands dedicated to production of its potato host *Solanum tuberosum*. Current fungicide based control strategies are far from being adequate and new ones are urgently needed. As *P. infestans* seems to be more related to the apicomplexan parasites than to true fungi, we believe that inhibiting the metabolic processes used to control human parasites might work to control this plant pathogen. In this study we investigated the dihydroorotase dehydrogenase DHODase, one of the key enzymes of the *de novo* pyrimidine biosynthetic pathway as a potential drugable target to develop novel control strategies. *In silico*, our preliminary molecular docking assays by MOLEGRO using three-dimensional (3D) homology modeled structures suggest that 6 of 7 parasite DHODase inhibitory compounds exert as well an inhibitory activity against the *P. infestans* enzyme. A counter selection strategy using the *S. tuberosum* model revealed that two promising inhibitory compounds seem to be species-selective and exert low or no inhibition over the hosts DHODase. Using the *P. infestans* N-terminally truncated DHODase expressed and purified recombinant protein that complemented a DHODase-deficient bacterial host we expect the to validate our *in silico* hypotheses.

Phylogenetic relations within *Aspergillus parasiticus* imply host adaptation and global transport of aflatoxin-producing fungi
N. GARBER (1), L. C. Grubisha (2), A. Ortega-Beltran (1), C. Probst (1), P. J. Cotty (2)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) USDA-ARS, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S39

Aflatoxin contamination of food and feed is an economic and health concern worldwide. In the U.S., active enforcement of maximum allowable aflatoxin levels for consumer protection can eliminate profit from crops including maize and cotton if aflatoxin is detected at concentrations of 20 parts per billion or greater. In countries with no aflatoxin-monitoring, human consumption of aflatoxins can result in aflatoxicosis with symptoms that range from immune system suppression to death. Two species in *Aspergillus* section *Flavi* are responsible for most aflatoxin contamination events: *A. flavus* and *A. parasiticus*. *A. flavus* communities span large geographic areas and associate with multiple crops, while *A. parasiticus* communities are discrete and commonly associated with few crops. In order to relate community structure to geography, host, and aflatoxin contamination risk, microsatellite loci and portions of multiple genes were amplified from *A. parasiticus* associated with peanut, maize, sugarcane and mixed cropping systems in North America, Asia and Africa. Phylogenetic analyses yielded multiple concordant gene trees whose topologies identify *A. parasiticus* lineages both associated with maize and peanut cropping worldwide, and a putative new species with an ancient, global and almost exclusive association with sugarcane (*Saccharum* cultivars). Population structure will be discussed as it relates to host preference, genetic diversity and geographic dispersal of *A. parasiticus*.

Comparative proteomic analysis of sugarcane response to infection by *Xanthomonas albilineans*, the causal agent of leaf scald
F. GARCES (1), J. W. Hoy (1), Z. Chen (1)
(1) Louisiana State University, Plant Pathology and Crop Phisiology Department, Baton Rouge, LA, U.S.A.
Phytopathology 100:S39

Leaf scald is a systemic, vascular disease of sugarcane that can cause severe yield reduction and the elimination of potential varieties. Although the use of resistant cultivars is the best control method, the molecular mechanisms of resistance in sugarcane are unknown. A comparative proteomic analysis to study differentially expressed proteins could provide a better understanding of the sugarcane resistance response during infection by X. albilineans. Single susceptible and resistant cultivars were selected according to disease severity, vascular infection and bacterial quantification by qPCR. After X. albilineans inoculation, leaf samples were collected during a time-course encompassing the response to initial and systemic infection. Total proteins were extracted and separated by two-dimensional electrophoresis. A relatively low number of proteins were differentially regulated during the initial infection of inoculated leaves in both susceptible and resistant cultivars. The number of up- and down-regulated proteins in the resistant cultivar increased during the systemic phase of infection 8 weeks after inoculation. To identify and determine possible functions of the proteins associated with the resistance response, they are being recovered for peptide sequencing. The preliminary proteomic analysis, vascular infection, and bacterial population evidence suggest that resistance is accomplished by mechanisms that limit systemic infection.

Control of *Fusarium* vascular wilt on carnation using soil biodisinfection
A. García (1), D. Palmero (2), M. de Cara (3), M. Santos (3), J. TELLO MARQUINA (4)
(1) Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA), Cadiz, SPAIN; (2) Technical University of Madrid, Madrid, SPAIN; (3) Almería, SPAIN; (4) Univ of Almeria, Almeria, SPAIN
Phytopathology 100:S39

The most important area for fresh cut flowers production in Spain is located in Cadiz province, attending 553 ha within a total of 1,164 ha in the whole country. Fusarium wilt (causal agent *Fusarium oxysporum* f. sp. *dianthi*) is the most important disease in carnation crops, meaning a limiting factor for production. Chemical soil fumigation together with the use of resistant cultivars against fungal infection used in the area did not provide a level of efficiency that makes carnation crop profitable. Efficacy of soil biodisinfection treatments using organic materials (C/N ~36) with and without polyethylene cover were evaluated for two consecutive years. Results showed that soil biodisinfection using compost of carnation and chrysanthemum (5kg·m-2) + hen manure (5kg·m-2) + solarization confers a suitable protection against the Fusarium vascular wilt during two years. Production and flower quality were significantly higher than any of the chemical fumigants tested (methyl bromide, 1,3 dichloropropene (1,3-D) + chloropicrin, metam-sodium) with and without polyethylene cover. Results also suggest that hen manure and standard high-density polyethylene film (HDPE) were both the key factor to the success of the biodisinfection. The proposed method using crops residues allows, not only the disease control, but also the reutilization of crop debris by using healthy and even diseased plants.

Population study on the Western Australian strains of *Sclerotinia sclerotiorum*
X. GE (1), K. Sivasithamparam (1), H. Li (1), M. Barbetti (1)
(1) The University of Western Australia, Crawley, AUSTRALIA
Phytopathology 100:S39

Sclerotinia disease, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most important yield-limiting and certainly the least controllable of diseases threatening oilseed and vegetable *Brassica* production world-wide, including Australia. In Western Australia, the disease is severe but the status of the pathogen population is largely unknown. Research is now being undertaken to 1) distinguish the variability within the pathogen population with respect to morphological characteristics, including cultural and physiological differences; 2) develop a set of host differentials to delineate pathotypes/strains of the pathogen using the inoculation and assessment methods developed by our team, 3) undertake molecular/ phylogenetic studies to compare strainal variation in Western Australia and elsewhere. One hundred and seventy isolates from oilseed and vegetable *Brassicas* and from some other broad-acre crop species from 17 sites in Western Australia were isolated and studied. Morphological studies indicated significant cultural and physiological differences among isolates, even within isolates sampled from the same site but from different individual host stems. A set of differential resistant response hosts was screened from ninety three genotypes of *B. napus* and *B. juncea* from China and Australia under semi-field conditions. Molecular and phylogenetic analyses are underway to determine the extent of genetic variation within the pathogen population.

Survey of soybean diseases in the Ohio River Valley Region of Ohio during 2009
K. GEARHART (1), D. Dugan (2), J. Grimes (3), L. Farley (4), J. Fisher (5), C. Burskey (4), A. E. Dorrance (1)
(1) Dept. of Plant Pathology, The Ohio State University, OARDC, Wooster, OH, U.S.A.; (2) Ohio State University Extension, Georgetown, OH, U.S.A.; (3) Ohio State University Extension, Hillsboro, OH, U.S.A.; (4) Ohio State University Extension, Owensville, OH, U.S.A.; (5) Ohio State University Extension, Waverly, OH, U.S.A.
Phytopathology 100:S39

Soybean yields in the Ohio River Valley Region of Ohio have been substantially less than other soybean producing regions of the state. The objective of this study was to determine what the production limitations are in the seven counties during 2009. A total of 132 plant and soil samples were collected at crop growth stages VE to V2 and R5 to R7 from 6 and 4 fields, respectively. Fields were sampled based on crop rotation practices and/or a history of low yields. Plant samples were transported back to the laboratory and symptomatic root tissue was plated onto selective media for both fungi and oomycete pathogens. Soil samples were processed for soybean cyst nematode (SCN) and soil analysis. *Pythium*, *Phytophthora*, *Fusarium*, *Macrophomina*, *Diaporthe*, *Sclerotinia*, and *Rhizoctonia* spp. were isolated from plant material. SCN was identified in 17 of the 38 locations and counts ranged from 0 to 3300 eggs/100cc soil. This is the first reported find of SCN for Pike Co. The next step is to further characterize the disease complexes that may be present, evaluate management strategies, and educate producers in this region.

Risk factors and modeling of powdery mildew occurrence on hop cones

D. H. GENT (1), J. L. Farnsworth (2), M. E. Nelson (3), G. G. Grove (3)
(1) USDA ARS NFSPRC, Corvallis, OR, U.S.A.; (2) Oregon State University, Corvallis, OR, U.S.A.; (3) Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S40

Powdery mildew of hop (caused by *Podosphaera macularis*) can substantially reduce crop cone yield and quality. Risk factors associated with the incidence (proportion) of diseased cones were identified and formalized in a linear mixed model based on published data from 12 fungicide efficacy trials conducted during 2000 to 2008. Models that included risk factors of disease incidence on leaves, rain, temperature, and fungicide timing during critical cone developmental stages explained 87 to 91% of the variability in observed incidence of diseased cones. Predictions of disease levels in 2009 with data sets collected independently of those used for model developed (validation data sets) were imprecise (R^2 = 0.55), in part because fungicide effects were not represented accurately. When the fungicide effect variable was revised, the model explained 74% of the variability in the observed incidence of diseased cones in the validation data sets. Sensitivity analyses indicated that the effect of fungicide application timing is substantial, and suggests appropriate timing of efficacious treatments is critical for minimizing levels of powdery mildew at harvest. Future research is planned to link the disease risk model to a crop damage function to inform and optimize late season management decisions for powdery mildew.

Identification and characterization of cucurbit powdery mildew in Florida

A. J. GEVENS (1), G. S. Maia (2)
(1) University of Wisconsin, Madison, WI, U.S.A.; (2) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S40

Cucurbit powdery mildew (PM) is a common and economically important foliar disease in vegetable production. Although cucurbit PM can be caused by two obligate pathogens, Podosphaera xanthii and Golovinomyces cichoracearum, P. xanthii has been most commonly identified. Recently, there has been an increase in occurrence and severity of cucurbit PM in FL, raising concern of a shift in the population. In this study, we identified and characterized single colony isolates of cucurbit PM from multiple sites, dates, and hosts. Two butternut squash fields (Live Oak and Citra, FL) were sampled during spring 2009 and the Citra site was again sampled in fall 2009. For comparison, additional cucurbit isolates were collected from SW and NE FL. For all 264 PM isolates from butternut squash from Live Oak and Citra, conidia were hyaline, ellipsoid-ovoid, in single chains per conidiophore, and had dimensions of 32-44 X 18-20 µm (N = 50) and footcells of 47-61 X 11-13 µm (N = 25). All isolates exhibited fibrosin bodies in immature conidia and conidia edges were crenate. Isolates from butternut squash and additional cucurbit hosts from varied locations and dates were subjected to multiplex polymerase chain reactions with species-specific primers for P. xanthii (S1/S2) and G. cichoracearum (G1/G2). With S1/S2, a specific PCR product (454 bp) was amplified from genomic DNA of all isolates. All FL 2009 PM cucurbit isolates were identified as P. xanthii.

Enhanced quality, value, yield, carbon capture and sustainability of switchgrass biomass by the improvement of root, microbe and soil interactions

S. GHIMIRE (1), K. Craven (1), S. Chaluvadi (2), J. Bennetzen (2), E. Worley (1), J. Yang (1), M. Udvardi (1), M. Keller (3)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) The University of Georgia, Athens, GA, U.S.A.; (3) BioEnergy Science Center, Oak Ridge, TN, U.S.A.
Phytopathology 100:S40

Sustainable production of switchgrass (*Panicum virgatum* L.) for biofuel will depend, in part, on maximizing nutrient acquisition and assimilation throughout the growing season as well as minimizing nutrient loss at harvest. Nutrient acquisition and uptake by plants can be enhanced by beneficial soil microbes as well as those existing endophytically within the roots of the switchgrass host. We have undertaken a comprehensive characterization of the microbes associated with the rhizosphere of planted switchgrass cultivars and those found within the healthy, surface-sterilized root systems of plants from natural habitat. High levels of microbial biodiversity were detected for both fungi and bacteria, and several strains have been isolated for evaluation of fitness effects on elite switchgrass cultivars. Dramatic differences in rhizosphere and endophyte microbial populations have been found to be a function of host genetics by analysis of different switchgrass cultivars, and mapping studies are now initiated to identify the host genes that determine microbial composition in and around the root. The evaluation of 31 switchgrass accessions for natural variations in nutrient-use and remobilization efficiencies of 20 different elements in shoots of field-grown plants at maturity and after senescence revealed that accessions differed in elemental composition in these two developmental stages. Implication of these findings in sustainability of switchgrass production will be presented.

Powdery scab effect on potato *Solanum tuberosum* and *S. phureja*

E. GILCHRIST (1), J. Soler (2), J. G. Morales (1), S. Reynaldi (1)
(1) Universidad Nacional de Colombia, Departamento Ciencias Agronómicas, Medellin, COLOMBIA; (2) Universidad Nacional de Colombia, Medellin, COLOMBIA
Phytopathology 100:S40

Spongospora subterranea f. sp. *subterranea* is the causal agent of the potato powdery scab, this microorganism infects young tissues. There is nor effective control method available for the disease neither resistance varieties, it was evaluated the influence of the cycle culture duration on the powdery scab effects over two potato species. The potato *Solanum tuberosum* variety Diacol Capiro is six months cycle culture duration and *Solanum phureja* variety Criolla Colombia is four months. These were planted in free and infect soil whit *S. subterranea*. Both species had galls at 100% of the roots in infected soil, those galls covered the 5% of the root surface in *S. tuberosum* and the 8% in *S. phureja.* There was no quantitative relation between the affected root surface with the length decrease, foliar dry weight and production. There were similar disease effects in both potato species, suggesting that there is not a susceptibility window related with the cycle culture duration. Next researches should focus in determinate if species or varieties with different development speed have any differential susceptibility to the disease, it should evaluate the powdery scab effects at the same sampling time.

***Trichoderma asperellum* T109 effect over *Spongospora subterranea* in potato field**

E. GILCHRIST (1), J. Pérez (2), H. A. Vargas (2), S. Reynaldi (1)
(1) Universidad Nacional de Colombia, Departamento Ciencias Agronómicas, Medellin, Antioquia, COLOMBIA; (2) Universidad Nacional de Colombia, Medellin, COLOMBIA
Phytopathology 100:S40

The aim of this research was to determinate the *T. asperellum* T109 effects over *S. subterranea* cystosori and over the powdery scab in potato *Solanum tuberosum* variety Diacol Capiro in field evaluating three doses. The removal of cystosori seems to be regulated by the concentration of the inocula, both for fault and for excess. It was determinate the effect of the *T. asperellum* T109 application on *S. tuberosum* plants without (control) and with 1082 ± 187 cysotosory/g soil of *S. subterranean.* In contaminated soil the plants were 26% less big and its production was 30% less, the *T. asperellum* T109 application reduce in 64% and in 54% the powdery scab effects in the plant growth and production, respectably. Future researches will have to determinate what factors limit the *T. asperellum* T109 activity in field.

Evaluation of fungicides on white mold of peanuts

S. GLUCKSMAN (1), C. R. Semer (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S40

Peanuts, found in foods such as candy bars, peanut butter, peanut brittle, and peanut flour, are also found in cosmetics, nitroglycerine, plastics, dyes, and paints. Peanut oil is also a commodity derived from this legume, and is responsible for approximately 4% of the global vegetable oil production at 4.93 million metric tons. Florida produced approximately 10% of the 5.15 billion pounds of peanuts produced in the United States in 2008. White mold causes an estimated 7% loss from disease on peanuts annually valued at $7 million. The purpose of this experiment was to evaluate the efficacy of several fungicides for the control of white mold (*Sclerotium rolfsii*) on peanuts. An alternating application of Bravo Weatherstik® 1.25pt/A (AFG) and Provost®

10.7oz/A (BCDE), treatment 10, was found to produce the highest yield at 4543.31lbs/Acre. Besides the control, the lowest yield was treatment 12 (Bravoweatherstik® 1.25pt/A (ABCDEFG)) at only 3645.97 lbs/Acre.

SSR-based genetic analysis of *Candidatus* Liberibacter asiaticus isolates from multiple continents

J. M. GLYNN (1), H. Lin (1), C. Chen (2), Y. Duan (3), L. Zhou (4)
(1) United States Department of Agriculture, Parlier, CA, U.S.A.; (2) Guangxi Citrus Research Institute, Guangxi, PRC PEOPLES REP OF CHINA; (3) United States Department of Agriculture, Ft. Pierce, FL, U.S.A.; (4) University of Florida, Ft. Pierce, FL, U.S.A.
Phytopathology 100:S41

Huanglongbing (HLB) is one of the most devastating citrus diseases that threaten citrus production worldwide. The causal agents are believed to be *Candidatus* Liberibacter spp. Although substantial efforts have been made toward detection of the presumed pathogens, information regarding to the pathogen's genetic variation, population structure, and epidemiological relationships is limited-- such data might provide useful insights into the evolutionary adaptations and host selection mechanisms utilized by this group of bacteria. The objective of this study is to identify the phylogenetic relationship between *Candidatus* Liberibacter asiaticus (Las) strains isolated from Florida, Brazil, India, and China. We used a panel of at least 8 simple sequence repeat (SSR) markers to infer phylogenetic relationships amongst HLB-associated Las strains from these four geographically- and ecologically-diverse regions. Our results indicate that Las strains from Florida share features with strains from three other regions of the world and provide insights into regions of the Las genome that may be associated with altered levels of pathogenicity.

Analysis of induction and establishment of dwarf bunt of wheat under marginal climatic conditions

B. J. GOATES (1), G. L. Peterson (2), R. L. Bowden (3), L. D. Maddux (4)
(1) USDA ARS, Aberdeen, ID, U.S.A.; (2) USDA ARS, Frederick, MD, U.S.A.; (3) USDA ARS, Manhattan, KS, U.S.A.; (4) Kansas State University, Topeka, KS, U.S.A.
Phytopathology 100:S41

Dwarf bunt caused by *Tilletia contraversa* has limited distribution due to essential climatic requirements; primarily persistent snow cover. The pathogen is a quarantine organism in several countries outside of the U.S.A., some of which may have marginal climate for the disease, including the People's Republic of China. To evaluate the risk of disease introduction, experiments were conducted in Kansas which is a climatic analog to the northern winter wheat areas of China. Four replicate 27 m^2 plots, planted with a susceptible cultivar, were inoculated at six rates ranging from 0.88 to 88,840 teliospores/cm^2. Three separate nursery sites were inoculated once, each site in a separate season, followed by replanting and examination for disease for 4 to 6 years afterward. Any diseased spikes produced were crushed and returned to the plots. Bunt was induced at trace levels at the three highest inoculation rates in two of the three nurseries. One nursery had no disease during all of six seasons. Disease carryover occurred during one year in one nursery at the highest inoculation rate, but no disease occurred in three subsequent seasons. In all nurseries, the disease eventually disappeared. A duplicate nursery planted in a disease conducive area showed the highest inoculum rate caused almost 100% infection. This research substantiates the critical importance of climatic conditions for establishment of this pathogen and contributes to pest risk assessment efforts.

Genetic diversity of the vegetable pathogen Phytophthora capsici in Argentina

D. J. GOBENA (1), J. Roig (2), J. Hulvey (1), K. Lamour (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.; (2) INTA EEA La Consulta, Mendoza, ARGENTINA
Phytopathology 100:S41

Phytophthora capsici is an oomycete plant pathogen that attacks solanaceous and cucurbit hosts worldwide. In the U.S., populations from single fields often exhibit extensive genotypic diversity. However, in Peru a single A2 mating type clonal lineage dominates over a wide area. Here we present data on 43 isolates of P. capsici recovered from pepper, paprika and pumpkin hosts in 2006, 2007, 2008 and 2009 from different regions in Argentina. All of the isolates were the A1 mating type. The isolates were assayed for population specific single nucleotide polymorphism (SNP) and AFLP profiles. The SNP's were identified by re-sequencing single copy genes in two isolates from the Argentina populations and were assessed for all isolates using high resolution DNA melting analysis. Our preliminary data indicates four unique genotypes with one clonal lineage comprising 86% of the population and two other clonal lineages with only two isolates. An overview of the marker development, application and resultant findings will be presented.

Cell cycle regulator *MoCDC15* is required for conidiation and pathogenicity in *Magnaporthe oryzae*

J. GOH (1), K. Kim (1), Y. Lee (1)
(1) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, SOUTH KOREA
Phytopathology 100:S41

Rice blast, caused by *Magnaporthe ozyzae*, is a serious constraint to rice production, and has emerged as an important pathosystem to uncover molecular mechanisms in plant-fungus interactions. Like other fungal pathogens, *M. oryzae* produces a massive amount of conidia, via conidiation for dissemination and exacerbation of the disease. However, relatively little is known about molecular mechanism of conidiation in *M. oryzae*. To better understand conidiation mechanism, we identified a conidiation-defective mutant, ATMT0225B6 ($MoCDC15^{T\text{-}DNA}$), from a T-DNA insertional mutant library. Molecular and bioinformatics analyses revealed that T-DNA was inserted in a gene encoding a Pombe CDC15 Homology (PCH) ortholog (*MoCDC15*) in ATMT0225B6. To unveil functional roles of *MoCDC15*, the same allele mutant ($\Delta MoCDC15^{T\text{-}DNA}$) was generated by a single step gene replacement strategy. Both mutants exhibited the same phenotype defects in conidiation, conidial germination, appressorium formation, and pathogenicity. Conidia of these mutants were in abnormal shapes with reduced adhesion ability to hydrophobic surface. All these phenotypic defects were recovered in the complemented strains (*Mocdc15c*). These results indicated that *MoCDC15* is essential for conidiation, infection-related development, and pathogenicity in *M. oryzae*.

DNA pyrosequencing to determine the influence of fallow period on soil microbial communities in the Bolivian highlands

L. GOMEZ (1), A. Jumpponen (1), M. A. Gonzales (2), J. Cusicanqui (3), C. Valdivia (4), P. Motavalli (4), M. Herman (1), K. A. Garrett (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) Fundacion PROINPA, La Paz, BOLIVIA; (3) Universidad Mayor de San Andres, La Paz, BOLIVIA; (4) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S41

In the Bolivian highlands (Altiplano; approx. 4000 masl), traditional fallow periods are being shortened in an effort to increase short-term crop yields, which may be at the expense of soil quality and plant health. Using 454-pyrosequencing and DNA-tagging, we characterized the response of the microbial community to (1) the length of fallow period and (2) the presence of *Parasthrephia* sp. and *Baccharis* sp. (both locally known as 'Thola'), considered beneficial to the maintenance of soil health in fallow fields in this region. 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 macronutrients, which supported more diverse fungal communities ($P < 0.001$). The presence of Thola after ten years of fallow had a positive effect on soil fungal diversity. Unexpectedly, the longer fallow periods were associated with lower fungal richness and diversity, perhaps because some fields with longer fallow periods were perceived by managers to have lower quality soils. Analyses of bacterial communities and fungal community composition are underway. Our results suggest that the drivers of microbial richness may be more complex than predicted by fallow period alone, and that plant cover may be important in conserving microbial communities.

Relationship between Citrus Variegated Chlorosis intensity and yield reduction

F. P. GONÇALVES (1), E. S. Stuchi (2), S. A. Lourenço (1), L. Amorim (1)
(1) Escola Superior de Agricultura Luiz de Queiroz / USP, Piracicaba, BRAZIL; (2) Embrapa Mandioca e Fruticultura / Estação Experimental de Citricultura de Bebedouro, Bebedouro, BRAZIL
Phytopathology 100:S41

Citrus Variegated Chlorosis (CVC), caused by *Xylella fastidiosa*, causes losses of around 120 million dollars/year in Brazil. CVC is present in Brazil since 1987, even though, the relationship between disease intensity and yield losses has never been established. The objective of this study was to determine this relationship. The incidence of branches with symptoms of CVC and the asymptomatic fruit yield (asymptomatic fruit production) were evaluated during three years (2006 to 2008), in orchard located at São Paulo State. The experiment design was in factorial randomized blocks (3 × 2). The treatments were: no irrigation, irrigated with 50% and 100% of crop evapotranspiration (ETc), combined with natural and artificial inoculation of *X. fastidiosa*. Each plot consisted of 6 plants, with 4 replication, total 144 trees (10-yr-old Natal sweet orange). The single plant method was used to quantify disease damage. For all treatments, the decrease of yield (asymptomatic fruit production) was associated with the increased of CVC incidence, described by the negative exponential model. The reductions of yield rates were similar for

all treatments (b = 0.014 to 0.019). Because of this, one single model can be used to describe the yield-CVC intensity relationship: y = (114. 07)*exp(0.017) *x), where y is yield (Kg of fruit asymptomatic) and x is disease incidence, $p < 0.01$ with $R^2 = 0.45$. According to the model, 10% of symptomatic branches lead to a reduction of 18.3 kg/plant. Supported by CNPq and Fundecitrus.

Morphological and molecular characterization of *Olpidium virulentus*, the fungal vector of the Macana virus disease in Colombia
C. GONZALEZ (1), D. L. Osorio (1), M. C. Cepero de García (1), L. Sastoque (1), C. Beltrán (1), A. M. Cotes (1)
(1) CORPOICA, Mosquera, Cundinamarca, COLOMBIA
Phytopathology 100:S42

Olpidium spp. is a fastidious root-infecting plant parasite whose eradication is difficult because of its condition as obligated root plant parasite as well as it having resistant structures. It is considered as transmission vector of virus such as Dianthovirus from the family of the Tombusviridae, which is a causal virus of the 'Macana' or necrotic streak disease of fique (*Furcraea* spp.). The Macana disease symptoms are characterized by necrotic stripes which appear on the infected plant leaves, followed by growth reduction and finally, total death. In order to to identify the *Olpidium* specie involved in the Macana disease in Colombia, extensive field surveys in three municipalities in the Cauca department, and microscopic studies of non-motile reproductive stage and motile zoospore was conducted using a contrast phase microscope. Fungal isolate was directly obtained by cultivation of motile zoospore from infected roots on lettuce plant. Fungal DNA was obtained from both zoospore and root plants, and a multiplex PCR was carried out using reported species-specific primers of three *Olpidium* spp. The microscopic analysis and PCR data indicated that fungal specie obtained from fique corresponded to *Olpidium virulentus*.

Genotypic diversity of *Phytophthora ramorum* in Canada
E. M. GOSS (1), M. Larsen (1), A. Vercauteren (2), S. Werres (3), K. Heungens (2), N. J. Grunwald (1)
(1) USDA ARS, Corvallis, OR, U.S.A.; (2) Institute for Agricultural and Fisheries Research, Merelbeke, BELGIUM; (3) Julius Kuehn Institute - Federal Research Centre for Cultivated Plants, Braunschweig, GERMANY
Phytopathology 100:S42

Characterization of the genetic structure and diversity of the sudden oak death pathogen, *Phythophthora ramorum*, in ornamental nurseries in the United States has shown that all three known clonal lineages of the pathogen are present. The most common clonal lineage in U.S. nurseries has been the NA1 clonal lineage, which has the wider distribution in the United States as a result of interstate shipments of infected nursery stock. British Columbia (BC), Canada is also known to have nursery infestations of *P. ramorum*, and shipments of infected plants between the United States and BC have occurred. We investigated the genotypic diversity of *P. ramorum* in BC nurseries and compared this population to U.S. and European nursery populations. All three of the *P. ramorum* clonal lineages were found among Canadian nursery isolates, but the most common was the NA2 lineage. The NA1 clonal lineage was found infrequently in comparison to the United States. The EU1 lineage was observed almost every year and shared multilocus genotypes with isolates from Europe and the United States. Appropriate markers for the characterization of the NA2 lineage are needed.

Fungal gene expression patterns during infection of field pea roots by *F. graminearum* and *F. avenaceum*
R. S. GOSWAMI (1), K. Chittem (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S42

Fusarium root rot of field peas has been a growing concern in the Midwestern production areas. Surveys conducted over the past three years demonstrated that species such as *F. avenaceum* and *F. graminearum*, previously associated with cereals in the U.S., were infecting roots of this major rotational crop. Information regarding the infection of legumes by these species is limited and our goal is to elucidate the mechanism of disease development through genomic analysis and pathogenicity studies. Fungal gene expression during infection of field pea roots by these two pathogens was evaluated as a part of this study. Over eleven thousand *F. graminearum* genes were detected, 6.57% of which are considered to be associated with cell defense, rescue and virulence. Initial comparisons suggest that 1606 *F. avenaceum* genes identified are similar to those expressed by *F. graminearum* during pea root infection. Genes common between crown rot and head blight in wheat and those associated specifically with crown rot were identified in this study in addition to genes involved in the trichothecene biosynthetic pathway. Analysis of fungal gene expression patterns, the ability of *F. graminearum* to produce mycotoxins on peas and their potential role in infection of this host will be presented.

Spatial and temporal analyses of *Plum pox virus* survey data
A. GOUGHERTY (1), R. Welliver (2), N. Richwine (2), F. W. Nutter (1)
(1) Iowa State University, Ames, IA, U.S.A.; (2) Pennsylvania Department of Agriculture, Harrisburg, PA, U.S.A.
Phytopathology 100:S42

Plum pox virus (PPV) was recently declared to be successfully eradicated in Pennsylvania, following the implementation of a statewide eradication program initiated in 2000. There were approximately 400 PPV-positive trees detected in 2000, however, the number of PPV-positive trees decreased exponentially during the following years until 2007, when no new PPV-positive trees were detected. The success of the eradication program can be attributed to an extensive annual state-wide survey and the removal of all susceptible hosts within 500 m of a PPV-positive tree. Using 10 years of survey data, a number of spatial analyses were performed at the block and homeowner scale to quantify the spatial and temporal dynamics of the pandemic. In 2000, positive blocks were found to be clustered for distances between 0.7 and 4.9 km. In later years, the distance to 50% of new PPV-positive blocks/homeowners (D_{50}) increased from 1.3 km in 2000 to 17.2 km in 2005. In 2006, the last year any PPV-positive blocks/homeowners were detected, the D_{50} decreased to 6.3 km. An approximate two year periodicity was found to exist arising from previous positive locations, suggesting that the location of a PPV-positive block/homeowner was having an impact on the health status of other *Prunus* blocks at least 2 years after being removed. This was further supported by a nearest neighbor analysis that revealed the time since removal of the nearest positive block/homeowner averaged to be 1.8 years.

Development of primers and probes for detection of Citrus *Candidatus* Liberibacter species by real-time PCR
A. GOVINDARAJULU (1), N. Choudhary (1), J. S. Hartung (2), A. L. Stone (3), V. D. Damsteegt (3), R. H. Brlansky (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) USDA-ARS, Beltsville, MD, U.S.A.; (3) USDA-ARS, Fort Detrick, MD, U.S.A.
Phytopathology 100:S42

Citrus huanglongbing (HLB) or citrus greening is the most threatening bacterial disease of *Citrus* spp. Three uncultured *Candidatus* Liberibacter spp. are usually detected by conventional PCR or real-time PCR using species specific primers and probes based on the *16S* rRNA gene. Recent molecular analyses suggest that the *rpo*B gene (encoding the β-subunit of RNA polymerase), is useful for bacterial identification. We report the design of a pair of degenerate primers and probe in the *rpo*B region and its application for the detection of the three Liberibacter species (*Ca*. Liberibacter asiaticus, *Ca*. L. africanus and *Ca*. L. americanus). In addition, a multiplex format was standardized and applied to detect all three species. This one step real-time PCR diagnostic method is reliable for the detection of Liberibacter infection regardless of species. This method also may be useful to detect unidentified Liberibacter species in citrus.

Soil application of neo-nicotinoid insecticides and acibenzolar-S-methyl for induction of SAR to control citrus canker on young citrus trees
J. H. GRAHAM (1), M. Myers (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S42

Soil application of systemic imidacloprid produces season-long control of citrus canker caused by *Xanthomonas citri* subsp. *citri*. This neo-nicotinoid insecticide induces systemic acquired resistance (SAR) measured as an increase in salicylic acid levels and expression of PR-2 gene in citrus leaves. Soil drenches of imidacloprid (Admire, Bayer Crop Science), thiamethoxam (Platinum, Syngenta Crop Protection), and the commercial SAR inducer acibenzolar-S-methyl (Actigard, Syngenta Crop Protection) were compared with copper hydroxide (CH, Kocide 3000, Dupont Crop Protection) applied as a spray at 21 da interval for canker control on foliage of 4-yr old 'Ray Ruby' grapefruit trees. Canker on each set of foliar flushes was assessed as the percentage of the leaves with lesions. All treatments significantly reduced incidence of foliar canker compared to the untreated check. Platinum and Actigard as soil drenches were as effective as previously tested drenches of Admire for sustained control of canker on young trees under epidemic conditions. Although the level of control did not match that of 11 contact sprays of Kocide 3000, SAR activity persisted season long. For both the 2008 and 2009 seasons, canker control with SAR inducers was highest for four applications of Actigard at 60 da interval. This treatment demonstrates the efficacy of repeated soil applications for maintaining SAR throughout the season.

Genetic variation and adaptation of *M. perniciosa* isolates in Bahia, Brazil
K. P. GRAMACHO (1), J. Pires (2), R. Moreira (3), U. V. Lopes (2)
(1) CEPLAC CEPEC SEFIT, Itabuna, Bahia, BRAZIL; (2) CEPLAC CEPEC SEGEN, Itabuna, BRAZIL; (3) UFR, Cruz das Almas, BRAZIL
Phytopathology 100:S42

The incidence of witches' broom disease of cacao (*Moniliophthora perniciosa*) in Bahia, Brazil has increased towards the main source of resistance: the Scavinas. It is hypothesized that this alterations is due to pathogen variability. Cross-infectivity of isolates of *M. perniciosa* from the Scavina's descendent was evaluated. The experiment was arranged as a randomized complete block design with 4 replicates of treatments that included four isolates of *M. perniciosa* derived from the Scavina's descendent (Sca6, THS1188, TSH565, TSH516) and two from other resistant (CCN10) and susceptible (SIC2) genotypes. Clones of Sca6, TSH1188, TSH565, CCN10, and SIC 23 (susceptible) were inoculated with 20-µL droplet of 2×10^5 basidiospores/mL per shoot. Sixty days after the inoculation day, plants were evaluated considering the variables SINT (disease incidence) and ID (disease index). There were significant differences among the *M. perniciosa* isolates. Most Scavina's descendent isolates were significantly more virulent than the isolate derived from the susceptible genotype. Moreover, based on molecular markers (RAPD), fungal isolates from resistant genotypes differed ($p < 0.05$) from the susceptible ones. Considering the increase in susceptibility in Scavina descendants, associated with the distinction of isolates from susceptible/resistant genotypes, it can be concluded that the increase in susceptibility is the result of the increase in frequency of strains capable of overcoming the resistance of Scavina.

The effects of temperature, humidity, and wounding on development of Phytophthora rot of cucumber

L. L. GRANKE (1), M. K. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S43

The effects of temperature (10, 15, 20, 25, 30, 35°C at >97% relative humidity) and relative humidity (<45%, ~60%, ~70%, ~80%, ~90%, and >97% at 25°C) on development of Phytophthora fruit rot of pickling cucumber were investigated in controlled growth chamber studies. In addition, the effect of wounding on size related resistance of pickling cucumber to *P. capsici* was characterized for three fruit sizes: 2.0 to 2.5 × 8 to 9 cm (small), 3.0 to 4.0 × 12.0 to 13.0 cm (medium), and >4.5 cm × >14 cm (large). No lesions developed on cucumbers incubated at 10°C, but lesions were observed on cucumbers incubated at all other temperatures tested. Lesion development was delayed for cucumbers incubated at 15°C. Lesion diameter and sporulation were greatest on cucumbers incubated at 25°C at 4 days post inoculation (dpi). Lesion development was greatest on cucumbers incubated at ≥80% relative humidity, but lesions formed on cucumbers incubated at all levels of relative humidity tested. Wounding was found to lessen size-related resistance in pickling cucumber. Lesion size was similar for all wounded cucumbers at 4 dpi regardless of fruit size. The smallest lesions were observed on unwounded large cucumbers. Sporangial production was greatest on wounded small and medium fruits. Fewer sporangia were produced on the large unwounded fruits than on any of the other cucumbers tested. Similar numbers of sporangia were produced on wounded large fruits and unwounded small and medium cucumbers.

Influence of environment on periodicity and concentration of airborne *Pseudoperonospora cubensis* sporangia in commercial cucurbit fields

L. GRANKE (1), M. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S43

Airborne concentrations of *Pseudoperonospora cubensis* sporangia were monitored from 2006 to 2009 in commercial cucurbit fields in four Michigan counties. An additional two counties were monitored for one or two growing seasons. Environmental conditions (temperature, relative humidity, leaf wetness, and rainfall) were recorded at select sites during the 2008 and 2009 growing seasons. The concentrations of airborne sporangia showed a marked diurnal periodicity with the highest concentrations between 600 and 1300 h. Airborne sporangial concentrations were positively related to average temperature and negatively correlated with both average relative humidity and leaf wetness at all of the sites monitored in 2008 and 2009. The results of this study may be used to improve the regional disease warning system, which already uses sporangial counts to guide fungicide applications.

Detecting *Sugarcane yellow leaf virus* in asymptomatic sugarcane leaves with hyperspectral remote sensing and associated leaf pigment changes

M. P. GRISHAM (1), R. M. Johnson (1), P. V. Zimba (2)
(1) USDA ARS, Houma, LA, U.S.A.; (2) Texas A&M - Corpus Christi, Corpus Christi, TX, U.S.A.
Phytopathology 100:S43

Sugarcane yellow leaf caused by *Sugarcane yellow leaf virus* (SCYLV) does not produce visual symptoms in most susceptible sugarcane plants until late in the growing season. High-resolution, hyperspectral reflectance data from SCYLV-infected and non-infected leaves of two cultivars, LCP 85-384 and Ho 95-988, were measured and analyzed on 13 July, 12 October, and 4 November 2005. Infection was determined by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Results from discriminant analysis showed that leaf reflectance was effective at predicting SCYLV infection in 73% of the cases in both cultivars using resubstitution and 63% and 62% in LCP 85-384 and Ho 95-988, respectively, using cross validation. Leaf pigments were extracted from leaf samples collected on 12 October and analyzed for chlorophylls and carotenoids concentrations. SCYLV infection influenced the concentration of several of the plant pigments including violaxanthin and b-carotene. Pigment data was effective at predicting SCYLV infection in 80% of the samples in the combined data set using the derived discriminant function with resubstitution, and 71% with cross validation. Developing technology to remotely detect SCYLV infections without a laboratory-based diagnostic technique would provide an efficient method to insure that seed cane is free of the SCYLV.

Evaluation of multiple management strategies for FHB barley

P. L. GROSS (1), S. M. Neate (2), R. Brueggeman (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) Toowoomba, AUSTRALIA
Phytopathology 100:S43

Fusarium head blight (FHB) has been a devastating disease for U.S. barley for over a decade. The disease causes kernel discoloration and the production of deoxynivalenol (DON). Genetic resistance, cultural practices, and fungicides have reduced the disease and toxin levels, but often not to levels required by the malting and brewing industry. We investigated the effects of resistant lines, fungicide treatment at heading, and crop rotations on FHB severity and DON accumulation in barley at Fargo, ND in 2009. Four six-row and four two-row barley lines with differential reactions to FHB were grown on ground planted the previous year to either wheat or soybean. Additionally, these plots had a fungicide treatment (Prosaro applied at 0.48 L/ha) at heading or were left untreated. FHB incidence, severity DON levels, yield and test weight were evaluated. Disease levels were very low in this study. Planting barley into soybean ground significantly increased yield and test weight for both barley types, across all lines, but disease parameters generally were not impacted by crop rotation. Fungicide treatment significantly reduced DON levels across the six-row lines, but not the two-row. Several-two row and one six-row line had significantly lower DON levels than more susceptible lines, across rotation and fungicide treatments. No significant differences were observed between interactions in six-row or two-row barley lines. This study will be repeated again in 2010.

Morphological and cytological modifications of *Gibberella zeae* germlings induced by mating pheromones and affinity-selected peptides

N. W. GROSS (1), J. E. Scholez (1), F. J. Schmidt (2), J. T. English (1)
(1) Division of Plant Sciences, University of Missouri, Columbia, MO, U.S.A.; (2) Department of Biochemistry, University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S43

Head blight of wheat is initiated in the spring by the homothallic ascomycete *Gibberella zeae*. This floral disease reduces grain yield but also results in the accumulation of various mycotoxins, the most notable among them being deoxynivalenol (DON). To reduce the need for fungicide treatment, wheat with robust resistance is necessary. However, in the absence of a dominant resistance gene, only defined types of partial resistance have been available to producers. Small peptides with specific fungistatic activity towards *G. zeae* are being developed as an alternative management strategy. These peptides, derived from combinatorial phage-display libraries and also from native *G. zeae* mating pheromone peptides, inhibit ascospore germination and induce abnormal germling phenotypes. Abnormalities induced by these mating pheromone peptides include extended periods of isotropic growth, apolar germtube emergence, and increased hyphal branching. After one day of peptide exposure, as many as 95% of ascospores exhibit abnormal morphological characteristics and these effects were observed over a wide range of peptide concentrations. Cytological analyses of ascospore and germling structure are in progress to better understand the basis of these morphological abnormalities. These data will advance our understanding of small-peptide inhibition of fungal spores which can be potentially exploited as a means of blight control.

Evolutionary relationships among *Aspergillus flavus* vegetative compatibility groups

L. C. GRUBISHA (1), P. J. Cotty (1)
(1) USDA-ARS, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S43

Aspergillus flavus is a fungal plant pathogen of many diverse crops including cotton, peanuts, maize, almond, and pistachio. During infection by *A. flavus*, crops are frequently contaminated with highly carcinogenic aflatoxins. *A. flavus* populations are composed of numerous vegetative compatibility groups

(VCGs), however not all VCGs produce aflatoxin. Identifying *A. flavus* isolates to VCG is a laborious and costly enterprise. We genotyped isolates using 19 microsatellite loci and the mating type locus, *MAT*, to elucidate evolutionary relationships among 20 VCGs. In addition, we assessed the utility of these molecular markers for initial VCG grouping. For the first objective, isolates from 20 VCGs were obtained from *A. flavus* populations associated with cottonseed in Texas and Arizona. For objective 2, 80 *A. flavus* isolates from maize in Texas were independently and blindly grouped into VCGs by both auxotroph complementation and microsatellite haplotype similarity. Results from these studies demonstrate that the same microsatallite markers can be used on *A. flavus* populations from both different geographic regions and different plant hosts. Relationships among isolates and VCGs will be discussed.

Characterization of the *occ* gene cluster required for the production of antifungal compound occidiofungin in *Burkholderia contaminans* strain MS14

G. GU (1), K. Chen (1), L. Smith (1), S. Lu (1)
(1) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S44

Occidiofungin produced by *Burkholderia contaminans* strain MS14 is an octapeptide dedicated to a broad range of antifungal activities of the bacterium. The 58-kb genomic fragment containing 18 open-reading frames (ORFs), named occidiofungin (*occ*) gene cluster, is required for occidiofungin production. Putative proteins encoded by five nonribosomal peptide synthetase genes (*occA – occE*) of the gene cluster were predicted to contain the catalytic modules responsible for the biosynthesis of occidiofungin. Transcription of all the ORFs identified in the region except ORF1 and ORF16 was regulated by both *ambR1* and *ambR2*, the LuxR-type regulatory genes located at the left border of the cluster. Sequence analysis revealed that the *occ* gene cluster, excluding ORF1 and ORF16, shared high similarity (99% nucleotide coverage and 91% identity) to an uncharacterized DNA region of *B. ambifaria* strain AMMD. The gene cluster was not found in other *Burkholderia* strains available in GenBank (nucleotide coverage < 24%). Analysis of G+C composition and prediction using "IslandPick" indicate that the *occ* gene cluster has possibly been horizontally transferred between bacteria. More importantly, the absence of the gene cluster in clinical strains of *Burkholderia* indicates that occidiofungin is not required for potential human pathogenesis. The findings have provided insights into the development of antifungal medicines or agricultural fungicides based on occidiofungin.

The role of fungal endophytes in the production of natural products in *Echinacea purpurea*

R. J. GUALANDI (1), K. D. Gwinn (1), B. H. Ownley (1), R. M. Auge (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 100:S44

Echinacea purpurea is a native herbaceous perennial with substantial economic value for its medicinal and ornamental qualities. Arbuscular mycorrhizae (AM) are symbiotic fungi that form relationships with plant roots and are known to enhance growth in the host. AM and other fungal endophytes often affect stress resistance and secondary metabolism in the host as well as the ecology of other endophytes in the plant. A newly emerging paradigm in sustainable biotechnology is the targeted use of fungal endophytes to enhance growth and secondary metabolism in crops. Many of the therapeutic compounds in *E. purpurea* could be affected by fungal colonization. This research tests the effects of intentional inoculation, of *E. purpurea*, with the AM fungi *Glomus intraradices* and *Gigaspora margarita* and the entomopathogenic fungal endophyte *Beauveria bassiana* (*Bb*). A series of greenhouse experiments tested endophyte colonization and changes in plant growth and phytochemistry. AM and *Bb* effectively colonized *E. purpurea* with some significant interactive effects on colonization. Consistent, substantial and significant increases in all growth parameters were observed in AM treatments. Substantial increases in P and N fertilization were necessary to produce AM and non-mycorrhizal samples of similar size. *Bb* had minor and inconsistent effects on some growth parameters and did exhibit some significant interactive effects with AM. Results of phytochemical analyses will be discussed.

Infectious RNA transcripts derived from cloned cDNA of *Calibrachoa mottle virus* (CbMV)

A. GULATI-SAKHUJA (1), H. Liu (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 100:S44

Calibrachoa is an important new horticultural plant both in Europe and the United States. Commercial reproduction of *Calibrachoa* plants and maintenance of genetic mother stock are done by means of vegetative propagation. A virus with spherical particles was isolated from *Calibrachoa* plants. The infected plant showed leaf mottling, chlorotic blotch and interveinal yellowing symptoms. The causal agent of this disease was named Calibrachoa mottle virus (CbMV). Based on the particle morphology, dsRNA profile and genome organization, CbMV was suggested to be a member of genus *Carmovirus*, family *Tombusviridae*. CbMV has a single stranded, positive-sense RNA genome of 3,919 nucleotides with five open reading frames (ORFs). To facilitate study of carmovirus proteins at molecular level, an infectious full-length cDNA clone of CbMV was constructed. RNA was extracted from purified virions and used for cDNA synthesis. Full length cDNA was constructed by using two overlapping fragments covering the entire CbMV genome. The T7 RNA polymerase promoter sequence was added upstream of 5'-UTR using PCR. Whole genome cDNA with fused T7 promoter sequence was cloned into pUC18 vector. Uncapped and capped *in vitro* RNA transcripts derived from the full-length cDNA clone of CbMV were infectious causing symptoms indistinguishable from those of the wild-type isolate on *Chenopodium quinoa*. The presence and validity of the progeny virus were verified by ELISA, RT-PCR and nucleotide sequencing.

Characterization of the HrpG and HrpX regulons of *Xanthomonas axonopodis* pv. *citri*

Y. GUO (1), N. Wang (2)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S44

Xanthomonas axonopodis pv. *citri* (Xac) is the causal agent of citrus canker and has a wide host range. The type III secretion system and effectors, which are essential for the pathogenicity of Xac, is controlled by two transcription regulators, HrpG and HrpX. We have been aiming at identification of the effectors of Xac. We postulate that effector genes will be control by HrpG and/or HrpX. In this work, we designed and conducted oligomicroarray analysis to characterize the regulon of HrpG and HrpX. Our analyses revealed that the expression of nearly 300 genes and 350 genes was significantly influenced by the mutation of the two transcription regulators HrpG and HrpX, respectively. The differentially expressed genes encode proteins belonging to 17 functional categories including potential effectors. Besides the known activities such as regulation of the expression of hrp and effector genes, microarray analyses also showed that HrpG and HrpX also influence flagellum synthesis, extracellular enzymes. Our results suggest that HrpG and HrpX not only regulate the expression of the type III secretion system and effector genes, but also influence genes in other functional categories during infection.

The gene *FvNoxR* of *Fusarium verticillioides* is required for its female fertility, normal hyphal ROS localization and full virulence on maize

L. GUO (1), G. A. Kuldau (1)
(1) Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S44

Fusarium verticillioides is a filamentous Ascomycete growing as a corn endophyte and causing corn ear rot, stalk rot and seedling blight. Fumonisin mycotoxins produced by this fungus pose a threat to animal health by causing fatal animal diseases such as equine leukoencephalomalacia and are associated with esophageal cancer of humans. Despite much understanding about fumonisin production and regulation it remains largely unknown how *F. verticillioides* causes disease and grows as an endophyte. Using genomic resources, we are studying the function of several *F. verticillioides* genes during fungal growth and disease development. An *in silico* search of the *F. verticillioides* genome using the *Epichloë festucae* NADPH oxidase regulator (NoxR) sequence as the probe revealed a hypothesized protein (FvNoxR) sharing 80% sequence similarity with NoxR. Deletion of the putative *FvNoxR* gene has shown pleiotropic effects. *ΔFvNoxR* exhibits little aerial hyphae, reduced conidiation and female sterility, although the mutant radial growth rate is similar to the wild type. Reactive oxygen species (ROS) level in *ΔFvNoxR* is comparable to the wild type *in vitro* but its localization differs. The mutant produces multiple hyphal branches on conidial germ tubes, a possible sign of lost hyphal polarity. Importantly, *ΔFvNoxR* has attenuated virulence on maize seedlings, ears and stalks. Efforts are underway to characterize the mutant *in planta* fungal growth and ROS level.

Fungicidal control of stem rust (*Puccinia graminis* f. sp. *tritici*) of wheat

V. GUPTA (1)
(1) South Dakota State University, Brookings, SD, U.S.A.
Phytopathology 100:S44

Stem Rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is one of most devastating diseases of wheat throughout the world. The management of stem rust with fungicides is of particular interest due to the development of highly virulent races of Pgt in eastern Africa (e.g. Ug99). Herein we present results from studies evaluating fungicidal activity against Pgt. First, inhibition of urediniospore germination was evaluated in vitro for most of the

commercially available wheat fungicides as single active ingredient dilution series. EC50 values were calculated and it was found that strobilurins were best at inhibiting spore germination followed by the newer triazoles. Second, application timing was evaluated in the greenhouse with fungicides applied 24 hr, 48 hr, and 96 hr before or after inoculation. All of the pre-inoculation treatments inhibited symptom 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 untreated control. Tebuconazole and prothioconazole were most effective in controlling stem rust. In 2009, no significant differences were measured, however symptom development was limited on the untreated plots due to below-average temperatures.

Analysis of *Mycosphaerella graminicola* populations from California, Indiana, Kansas and North Dakota with mating type and SSR markers

S. GURUNG (1), S. B. Goodwin (2), M. Kabbage (3), W. W. Bockus (4), T. B. Adhikari (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Crop Production and Pest Control Research Unit, Purdue University, West Lafayette, IN, U.S.A.; (3) Texas A & M University, College Station, TX, U.S.A.; (4) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S45

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is one of the most important foliar diseases of wheat. Genetic diversity of 333 isolates of *M. graminicola* collected from spring (California, North Dakota) and winter wheat (Indiana, Kansas) was analyzed for mating type and 17 SSR markers. The Indiana, Kansas, and North Dakota populations were further subdivided into two, six, and two subpopulations, respectively according to sampling sites and years of collection. Both mating types were equally distributed in clone-corrected populations from Kansas, Indiana and North Dakota, while mating type frequency deviated from a 1:1 ratio in the California population. Gene diversity values for the California, Indiana, Kansas, and North Dakota populations were 0.44, 0.53, 0.40, and 0.57, respectively. No evidence of linkage disequilibrium was observed in all populations or subpopulations analyzed. High gene flow was observed between the California and Kansas (Nm = 15.914) and Indiana and Kansas (Nm = 16.89) populations. Analysis of molecular variance showed that most genetic variation (> 82%) was within populations and subpopulations; less than 18% occurred between the populations or subpopulations. These results indicate that frequent sexual recombination occurs in *M. graminicola* populations in spring and winter wheat. Furthermore, all geographically separated populations of *M. graminicola* are genetically similar, suggesting a panmictic structure.

Genome wide association mapping of resistance to tan spot and spot blotch in spring wheat

S. GURUNG (1), J. M. Bonman (2), M. Acevedo (2), T. B. Adhikari (1)
(1) North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, U.S.A.
Phytopathology 100:S45

Tan spot, caused by *Pyrenophora tritici-repentis*, and spot blotch, caused by *Cochliobolus sativus*, are destructive diseases of wheat worldwide. Studying the inheritance of resistance to these diseases using bi-parental mapping populations is time consuming and, in addition, the complex inheritance and partial effects of the resistance makes conventional breeding difficult. Molecular markers linked to resistance genes could facilitate resistance breeding. The main objective of this study was to utilize association mapping to identify molecular markers associated with tan spot and spot blotch resistance. A total of 582 accessions of spring wheat landraces of diverse origin from the USDA National Small Grain Collection were assessed for resistance to tan spot (race 1 and race 5) and spot blotch at seedling stage during 2009 and 2010 in a greenhouse at NDSU. Diversity Arrays Technology (DArT) marker-based linkage analysis for quantitative trait analysis (QTL) of disease resistance loci is in progress. Whether the newly identified genes/alleles are novel or identical to those previously reported in wheat genome will be discussed.

Changes in flavonoid biosynthetic pathway genes and anthocyanins due to virus infection in grapevine (*Vitis vinifera* L.) leaves

L. R. GUTHA (1), L. F. Casassa (1), J. F. Harbertson (1), R. A. Naidu (1)
(1) Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S45

Symptoms of grapevine leafroll disease in red-fruited wine grape (*Vitis vinifera* L.) cultivars consist of green veins and red and reddish-purple discoloration of inter-veinal areas of leaves. In this study, reverse-transcription quantitative PCR was used to compare the expression of flavonoid biosynthetic pathway genes between symptomatic leaves infected with *Grapevine leafroll-associated virus-3* and uninfected green leaves in cv. Merlot. Using two constitutively expressed reference genes as the most invariant internal controls, we normalized the expression levels of candidate genes in virus-infected and uninfected leaves. The expression levels of different genes ranged from two- to fifty-fold increase in virus-infected leaves when compared to uninfected leaves, with *CHS3*, *F3'5'H*, *F3H1*, *LDOX*, *LAR1* and *MybA1* showing greater than 10-fold increase. HPLC profiling of anthocyanins extracted from infected and uninfected leaves indicated the presence of cyanidin 3-glucoside and malvidin 3-glucoside only in virus-infected, symptomatic leaves. Our results also showed significantly higher levels of two flavonols in virus- infected leaves than in uninfected leaves, with quercetin followed by myricetin being the predominant compounds. These results suggested modulation of anthocyanin pathway genes towards de novo synthesis of certain classes of end -products contributing to phenotypic expression of disease symptoms.

Novel detection of *Ralstonia solanacearum* race 3 biovar 2 using magnetic capture hybridization and real time-PCR

Y. HA (1), T. P. Denny (1), M. A. Schell (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S45

Ralstonia solanacearum race 3 biovar 2 (R3bv2), a quarantined pathogen not present in the U.S., is a soil-borne bacterium that causes lethal wilting of potato, tomato, geranium and many weeds. Rapid, robust and sensitive detection methods are needed to maintain exclusion of R3bv2. Standard and real time-PCR (RT-PCR) can be used for specific identification of cultured R3bv2 cells, but compounds in soil and plants inhibit amplification when sampling directly from these sources, resulting in false negatives. To circumvent this problem, we developed paramagnetic beads coated with biotinylated single-stranded DNA 'capture probes' that anneal to R3bv2-specific target sequences. Magnetic capture hybridization (MCH) using these beads was then used to purify and enrich R3bv2 DNA for subsequently detection by RT-PCR. Control experiments showed that the RT-PCR detection threshold was 10-fold higher before MCH than after MCH both for R3bv2 genomic DNA (10 fg/µl vs. 1 fg/µl) or R3bv2 bacteria suspended in water (10^3 cells/ml vs. 10^2 cells/ml). More importantly, when R3bv2 cells were suspended in 1% soil solution RT-PCR failed, whereas MCH followed by RT-PCR detected as few as 10^3 cells/ml. Similarly, amplification failed when R3bv2 cells were suspended in 6% potato extract, but use of MCH prior to RT-PCR detected down to 50 R3bv2 cells/ml. Therefore, by removing inhibitory compounds present in soil and plant samples, MCH should greatly enhance the utility of RT-PCR for detecting R3bv2.

Non-host resistance response at the nucleosome level

L. HADWIGER (1)
(1) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S45

In non-host resistance (innate immunity) the activation of an assortment of pathogenesis- related (PR) genes is responsible for slowing the growth of the invading pathogen. In the interaction, between pea endocarp and *Fusarium solani* f. sp. *phaseoli*, total resistance develops within 6 h and is associated with PR gene activations being initiated within 2 h. The mechanisms involved mirror those recently observed in animal systems and may result from alterations or remodeling of chromatin. The PR gene DRR206 activation occurs as there is an ubiquitination/reduction of histones H2A/H2B and a reduction in the architectural transcription factor, HMG A. The pea RNA polymerase and these nuclear proteins can be located within the region of the DRR206 promoter DNA and subsequently within the open reading frame at 2 and 4 h pi with chromatin immunoprecipitation (ChIP) analyses. Also there is a simultaneous reduction in the HMG A in this region. The RNA polymerase complex appears to be in place but "paused" at the promoter and subsequent movement through nucleosomes is likely reinitiated by the transient disassembly of histones H2A/H2B from DNA and in its wake a reformation of these and other nuclear components -- all occurring within sensitive regions of the pea chromosome.

Fine scale genetic structure of flowering dogwood in the Great Smoky Mountains National Park

D. HADZIABDIC (1), B. M. Fitzpatrick (1), X. Wang (2), P. A. Wadl (1), T. A. Rinehart (3), B. H. Ownley (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.; (2) Texas A&M - Texas AgriLife Research and Extension Urban Solutions Center, Dallas, TX, U.S.A.; (3) USDA-ARS, Southern Horticultural Laboratory, Poplarville, MS, U.S.A.
Phytopathology 100:S45

Flowering dogwood (*Cornus florida* L.) populations have experienced severe declines caused by dogwood anthracnose in the past three decades. Mortality has ranged from 48 to 98%, raising the concern that genetic diversity of this native tree has been reduced significantly. However, the response of each species to habitat disturbance may differ greatly depending on their biological

attributes, particularly pollen and seed dispersal ability. Nineteen microsatellite loci were used to evaluate the level and distribution of genetic variation throughout Great Smoky Mountains National Park (GSMNP). Significant genetic structure at both landscape and local levels were found. We infer that two genetic clusters exist within the park, mostly separated by the main dividing ridge of the Great Smoky Mountains. The differentiation is statistically significant, but subtle, with gene flow evident through low-elevation corridors. Even accounting for this structure, we observed heterozygote deficiency across all loci, implying nonrandom mating at a finer scale. This pattern of variation implies that pollination occurs primarily between related individuals despite wide dispersal of seeds.

Application rate and interval impact the efficacy of Heritage 50WDG fungicide for the control of Alternaria leaf spot on marigold

A. K. HAGAN (1), J. R. Akridge (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Brewton Agricultural Research Unit, Brewton, AL, U.S.A.
Phytopathology 100:S46

Heritage 50WDG at 0.04, 0.08, and 0.16 g ai/l when applied at 2-, 3-, and 4-wk intervals was compared with Daconil Weather Stik 6F and Eagle 40W for control of Alternaria leaf spot (*Alternaria tagetica*) in field-grown marigold in Alabama. While the French Dwarf marigold (*Tagetes patula*) 'Little Hero' was used in 2001, the American marigold (*T. erecta*) 'Discovery Yellow' was planted in the remaining study years. Fungicides were applied to run-off with a CO_2-pressurized sprayer from June 6 to September 1, 2001; April 30 to August 2, 2002; April 30 to September 17, 2003; and May 14 to September 10, 2004. When applied at 2-wk intervals, all rates of Heritage 50WDG gave better disease control in all years than Daconil Weather Stik 6F or Eagle 40W, which showed no activity against this disease. In two of three years, Daconil Weather Stik 6F applied weekly decreased disease severity compared with the non-fungicide treated control, but when applied at 2-wk intervals, disease levels were similar to the non-fungicide treated control in two of four years. Alternaria leaf spot intensified when Heritage 50WDG rates declined from 0.16 to 0.04 g ai/l and intervals increased from 2- to 4-wk. At 0.04 g ai/l, disease control with Heritage 50WDG was better at the 2- than at the 3- and particularly 4-wk schedule. Regardless of the level of disease, Heritage 50WDG at 0.08 and 0.16 g ai/l when applied every 3-wk gave effective control of Alternaria leaf spot on marigold.

Yield response and control of crown rust on oats with fungicides

A. K. HAGAN (1), M. B. Pegues (2)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Gulf Coast Resarch and Extension Center, Fairhope, AL, U.S.A.
Phytopathology 100:S46

Efficacy of selected fungicides for the control of crown rust was assessed on the rust susceptible oat variety 'Coker 227' in 2008 and 2009. The experimental design was a randomized complete block with four replications. Fungicides were applied with CO2-pressurized back pack sprayer at full flag leaf extension (GS 9) and/or head extension (GS 10.5). Crown rust was rated on a 0 to 10 scale where 0 = no disease, 1 = 1 to 10% leaf area symptomatic to 10 = flag leaf dead. In both years, all fungicide treatments had lower rust ratings than the control. In 2008, best rust control were obtained with two applications of Tilt 3.6E and Stratego 2.08E, while single applications of 3.1 and 6.2 fl oz/A Quadris 2.08SC were less ineffective. Highest yield gains were obtained with Tilt 3.6F, Stratego 2.08E, Headline 2.09E, and Quilt 1.67E. For 2009, single applications of Tilt 3.6E, Stratego 2.08E, Headline 2.09E and Quadris 2.08SC at GS 10.5 but not GS 9 gave the same level of rust control as two the two application programs with the same fungicides. Despite differences in rust severity, yields with the two applications of Tilt 3.6E, Stratego 2.08EC, Headline 2.09E at 6 fl oz/A, and Quadris 2.08SC were similar to that with a single GS 9 or GS 10.5 application of the same fungicide. Yield gains up to 46 and 33 bu/A were seen with fungicides in 2008 and 2009, respectively, for a net income increase of up to $145 to $203 per acre for seed oats valued at $5.30/bu.

Genetic diversity of Iranian *Fusarium oxysporum* f. sp. *ciceri* by random amplified polymorphic DNA or vegetative compatibility groups

S. HAGHIGHI (1)
(1) Islamic Azad University, Damghan Branch, Tehran, IRAN
Phytopathology 100:S46

Fusarium wilt of chickpea is a devastating disease in chickpeas growing in different regions of Iran. This fungal disease is caused by Fusarium oxysporum f. sp. ciceri and this pathogen can reduce yield by 15%. In order to study genetic diversity of this pathogen, thirty isolates of Fusarium oxysporum f. sp. ciceri were selected from different origins of Iran. In vitro pathogenicity tests of the isolates were performed using a root-dip assay and isolates were classified into three categories of highly, moderate and weakly virulent groups. Genetic diversity was analysed based on RAPD-PCR using ten random primers. Cluster analysis of DNA fragments were performed using MVSP software and a UPGMA method with jaccard coefficient. Results of RAPD-PCR showed a high genetic diversity among the isolates and clustered them into 17 groups. There was a correlation between RAPD groups and geographical localization of the isolates. The basis for VCG assignment was a complementation reaction between nit1 or nit3 and nitM mutants on minimal medium. Pairs of isolates which exhibited vigorous site of the two nit mutant-mycelia- indicating the formation of heterokaryon-were determined as vegetatively compatible and by using this method; the isolates were classified into eight groups. In conclusion, the results of isolate classification by RAPD and VCG are similar but, the RAPD technique is more efficient than VCG method and a population genetic analysis can be performed with greater precision.

Spread of huanglongbing through citrus and citrus relatives in retail nurseries and garden centers

S. E. Halbert (1), K. L. Manjunath (2), C. Ramadugu (3), R. F. LEE (2)
(1) Florida Division of Plant Industry, Gainesville, FL, U.S.A.; (2) USDA ARS National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, U.S.A.; (3) Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, U.S.A.
Phytopathology 100:S46

Huanglongbing (HLB or greening) was first found in Florida in 2005, has spread to all the citrus growing counties in the state. Infected nursery trees and psyllid vectors carrying the HLB associated bacteria need to be avoided for successful management of the disease. In the present study, over 1200 psyllid (*Diaphorina citri*) samples were collected from plants for sale in retail centers and garden centers in Florida over a period of four years and tested for the presence of HLB associated "*Candidatus* Liberibacter asiaticus" (LAS) by real time PCR. 5–12% of psyllid samples tested in different years were positive for LAS. About 8% of psyllid samples collected from citrus, 10% from *Murraya paniculata* and 6% from other plants were positive for LAS. *M. paniculata* is a preferred host of *D. citri* and is used widely as an ornamental plant in Florida. Presence of LAS positive samples in counties having no commercial citrus provides additional evidence for the role of retail nurseries and garden centers in spreading HLB throughout the state of Florida. The importance of regulation to prevent both the psyllids and the HLB associated Liberibacters in retail nurseries in regions where the disease has not yet established will be discussed.

Assessment of agreement between apple scab and fire blight forecasts based on SkyBit predictions and on-site weather records

N. O. Halbrendt (1), J. W. Travis (1), H. K. NGUGI (1), J. Russo (2)
(1) Penn State University, Biglerville, PA, U.S.A.; (2) ZEDX, Inc., Bellefonte, PA, U.S.A.
Phytopathology 100:S46

SkyBit is a site-specific electronic weather service that delivers daily weather and pest management information to fruit growers in the mid-Atlantic region. The goal of this project was to assess the quality and reliability of the disease forecast information delivered to growers via SkyBit. We evaluated weather data and disease infection predictions delivered via SkyBit in comparison with data collected on-site at the Penn State Fruit Research & Extension Center, Biglerville, Pennsylvania. Overall, forecasts of scab infection periods based on SkyBit remote-sensed data showed significant agreement with predictions from a Spectrum Technologies model using on-site data ($\chi^2 = 2.0$; $P = 0.157$, based on McNemar's test), and weakly agreed with forecasts based on the Mills Table scab model ($\chi^2 = 3.6$; $P = 0.059$). Fire blight infection predictions based on SkyBit (with '++' counted as an infection period) did not agree with MaryBlyt predictions ($\chi^2 = 6.4$; $P < 0.011$). However, when the high risk of infection events (H) from the MaryBlyt model were counted as infection periods, predictions based on the SkyBit fire blight model agreed with MaryBlyt forecasts ($\chi^2 = 0.333$; $P = 564$). Estimates of daily rainfall from SkyBit closely agreed ($r = 0.882$; $P < 0.001$) with on-site records but SkyBit under-estimated the actual amounts. We conclude that SkyBit delivers reliable data for predicting scab infection periods and daily rainfall, but it tends to overestimate fire blight infection periods.

Molecular interactions determining broad-spectrum partial late blight resistance in potato

D. HALTERMAN (1), Y. Chen (2)
(1) USDA/ARS, Madison, WI, U.S.A.; (2) Madison, WI, U.S.A.
Phytopathology 100:S46

The potato late blight resistance (*R*) gene *RB* (*Rpi-blb1*) belongs to the valuable class of plant *R* genes that confer resistance to a broad spectrum of pathogen isolates. RB protein recognizes the presence of members of the *Phytophthora infestans* effector family IPI-O to elicit resistance. We have studied *IpiO* diversity from 40 different *P. infestans* isolates collected from Guatemala, Thailand, and the United States. We have found that all of the

isolates contain IPI-O variants that can be recognized by RB. However, some of these isolates contain an extraordinarily large number of variants. A few isolates also contain an IPI-O variant (IPI-O4) that is not recognized by RB. Isolates containing IPI-O4 are able to overcome resistance in RB-containing potato leaves to cause significantly more disease than isolates that do not contain IPI-O4, even when other IPI-O proteins are present. We show that the presence of IPI-O4 blocks the ability of RB to recognize the presence of other IPI-O variants through direct interaction with the resistance protein, thereby preventing programmed cell death related to the resistance response.

Identification of novel regulatory genes of *Burkholderia glumae* for virulence factors

J. HAM (1), I. Barphagha (1), H. Karki (1), B. Shrestha (1), R. Melanson (1), R. Chen (1)
(1) Louisiana State University AgCenter, Baton Rouge, LA, U.S.A.
Phytopathology 100:S47

Burkholderia glumae is the major causative agent of bacterial panicle blight of rice. Symptoms of this disease include panicle blight, sheath rot and seedling blight. The phytotoxin, toxoflavin, and lipase are the two most important virulence factors of this pathogen. *B. glumae* also produces extracellular polysaccharides (EPS), which play an important role in bacterial pathogenesis in many plant or animal pathogenic bacteria. The quorum-sensing mechanism involving acyl-homoserine lactones is known to be a major regulatory system, which governs the production of virulence factors and the motility of *B. glumae*. In an attempt to identify additional regulatory genes of this pathogen for virulence, the *B. glumae* 336-gr1 genome was randomly mutagenized with a mini-Tn*5* derivative, mini-Tn*5*gus, and the mutants showing altered phenotypes in the production of known virulence factors were screened. From more than 20,000 random mutants, 56 mutants have shown reduced or increased production of toxoflavin, lipase or EPS. As the complete DNA sequence of the *B. glumae* BGR1 genome is publicly available, the genes mutated with mini-Tn*5*gus could be easily identified by sequencing the flanking regions of the transposon. From this study, several novel regulatory elements for the *B. glumae* virulence factors have been identified. Currently, we are investigating the functions of these newly discovered regulators on the expression of various virulence genes and the bacterial pathogenesis of *B. glumae.*

Over-expression of a maize WRKY transcription factor and its effect on the responses of Arabidopsis to biotic and abiotic stresses

P. HAN (1), Y. Xie (2), Z. Chen (2)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.; (2) Baton Rouge, LA, U.S.A.
Phytopathology 100:S47

WRKY proteins are comprised of a large family of transcription factors identified specifically from the plant kingdom. WRKY transcription factors contain one or two conserved WRKY domains. Evidence is showing that the transcription of WRKY gene plays important roles in many biological processes, such as senescence, abiotic stress and pathogen- triggered signal transduction cascades in numerous plant species. In this study, we selected one maize WRKY transcription factor (PTZm 631) that showed a significant upregulation in response to *Aspergillus flavus* infection than other maize WRKY transcription factors in a preliminary field inoculation study. Two over-expression vectors of this WRKY transcription factor under the control of d35S and ZmPR10 promoters (pCambia 1302-d35S-WRKY and pCambia 1302-P_{ZmPR10}-WRKY) were constructed. After transformation into Arabidopsis, homozygous transgenic plants were selected and the *WRKY* expression was determined using real time PCR. We are currently testing the responses of these transgenic Arabidopsis plants over-expressing *WRKY* to abiotic stresses including SA, H_2O_2, NaCl, ABA, KT, heat, and biotic stress such as *Pseudomonas syringae*.

Study on the genetic diversity within Phytophthora capsici with nuclear, mitochondria and SNPs markers in New Mexico

S. Hanson (1), M. PEIMAN WILLIAMS (1)
(1) New Mexico State University, Las Cruces, New Mexico, U.S.A.
Phytopathology 100:S47

Phytophthora species are devastating plant pathogens in both agricultural and natural environments. Phytophthora capsici is a severe pathogen on chile peppers grown in the desert southwest where it can cause up to 100% losses in severely affected fields. Pathogenicity and phenotypic studies have indicated a high level of diversity among P. capsici strains isolated from chiles in the desert Southwest. Prior work with several commonly used phylogenetic molecular markers such as ITS regions, B-tubulin, LUS, and Cox-II showed that these markers were uninformative as they were unable to distinguish strains of P. capsici based on location, time, or phenotypes such as pathogenicity. The development of informative molecular markers for distinguishing different strains of P. capsici would be useful for resistance breeding and epidemiological studies and is the long term goal of this project. Herein we report the evaluation of several single-nucleotide polymorphism (SNP) markers for their utility as molecular markers that are predictive of P. capsici phenotypic diversity. Ongoing studies suggest that several of these markers may be useful as markers to identify different strains of P. capsici.

Sugar beet seedling damping-off in Michigan

L. E. HANSON (1), J. McGrath (1)
(1) USDA ARS, East Lansing, MI, U.S.A.
Phytopathology 100:S47

Seedling damping-off is a constraint to sugar beet production, particularly in the humid eastern U.S. growing region. On average, only 60% of the seed planted in Michigan produces a final plant stand. A large part of this loss has been attributed *Aphanomyces cochlioides*, but little screening has been done. To improve understanding of damping-off, seedlings with symptoms were collected from commercial beet fields and research plots in 2008 and 2009. Hypocotyls and roots were surface disinfested, cut into sections, and plated on potato dextrose agar with antibiotics to restrict bacterial contamination or in sterile distilled water with sterile millet seeds to bait for oomycetes. Hyphal tip transfer or single spore isolation were used to obtain pure cultures. Morphological and molecular methods were used to identify and characterize isolates. *Rhizoctonia solani* was the most commonly isolated pathogen in 2008, followed by *Fusarium oxysporum*, *A. choclioides*, *Phoma betae* and *Pythium* spp. in the order listed. In 2009, *A. cochlioides* was the most commonly isolated pathogen, followed in decreasing frequency by *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani* and *Phoma betae*. Isolates of all the above genera were pathogenic on beet seedlings in the greenhouse. The *R. solani* isolates collected included AG 4, AG 2-2 IV and AG 2-2 IIIB. Thus, in addition to *Aphanomyces*, a complex of seedling diseases contribute to stand problems in Michigan.

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

W. HAO (1), C. Hong (1)
(1) VPI & State University, Virginia Beach, VA, U.S.A.
Phytopathology 100:S47

Plant pathogens, especially *Phytophthora* species in re-circulating irrigation water, present significant health risks to floral crops. One of current technologies for water decontamination is heat treatment, which is 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 as recommended in the current protocols. Specifically, we investigated the effect of water temperature on survival of chlamydospores and oospores using two of the most destructive species, *P. nicotianae* and *P. pini*. Oospores of *P. pini* did not survive after 12 h of heat treatment at about 42°C, and the majority of chlamydospores of *P. nicotianae* did not survive 24 h at the same temperature. These results suggest that water temperature for heat treatment may be lowered substantially from 95°C without sacrificing efficacy. This research is being expanded to include other stages of the life cycle of *Phytophthora* species and other major groups of pathogens as well as trials in greenhouse settings.

Characterization of *Salmonella enterica* genes, STM0978 and STM0693, with a role in plant colonization

L. HAO (1), J. Barak (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 100:S47

Salmonellosis outbreaks caused by consumption of produce are increasing. The causal agent of the disease, Salmonella enterica, is not a plant pathogen, but it can utilize specific molecular mechanisms to attach and colonize plants rather than passive contamination which can be rinsed away. In our study, two *S. enterica* serovar Typhimurium function unknown genes were studied, STM0978 and STM0693. In individual bacterial strain inoculation experiments, *ΔSTM0978* was defective in alfalfa sprout colonization at 48 h, with 2 logs reduction in population compared to the wild type. *ΔSTM0693* had no significant difference from the wild-type. However, in a co-inoculation experiment with *ΔSTM0693* and the wild type, *ΔSTM0693* had a significant lower population than the wild-type with 1 log reduction. Such defective phenotypes were not simply due to a growth defect, since growth of both mutants had no obvious difference from the wild type in 48 h alfalfa exudates. Further examination of *ΔSTM0978* found it defective in growth in M9 minimal media, swimming, and swarming compared to the wild-type. The function of both STM0978 and STM0693 is still underway. We are interested in targeting the essential compounds that are needed by the pathogen to survive in plants. Characterization of such genes will provide critical

understanding of the pathogen survival mechanism outside its warm-blood host and help to develop useful strategies to prevent plant contamination in the future.

Oxidized forms of silver as safe, effective seed treatments
M. W. HARDING (1), D. A. Sowa (2), R. J. Howard (3), M. E. Olson (2)
(1) Innovotech Inc., Brooks, AB, CANADA; (2) Innovotech Inc., Edmonton, AB, CANADA; (3) Alberta Agriculture and Rural Development, Brooks, AB, CANADA
Phytopathology 100:S48

Metallic fungicides have a long history of agricultural use, beginning with copper in the late 1800's in France for control of grape downy mildew. Silver, on the other hand, was not registered as a pesticide until 1954 and, at the time of this report, no silver-based fungicides were currently registered for agricultural use in North America. Innovotech Inc. has demonstrated that highly oxidized, silver-based compounds are excellent seed treatment compounds for eradicating seed-borne bacteria and fungi. For example, control of seed-borne bacterial blights on dry beans caused by *Pseudomonas syringae* pv. *syringae*, P. *syringae* pv. *phaseolicola*, *Xanthomonas axonopodis* pv. *phaseoli* and bacterial wilt caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* can be readily controlled or eradicated when seeds are coated with a 0.5% aqueous solution of oxysilver nitrate. Furthermore, silver-based seed treatments are effective on other crops, including soybean, tomato, ginseng and potato. These silver-based seed treatments are manufactured as wettable powders, which are effective at very low concentrations, i.e. 10 to 100 times lower than copper. Silver powders are compatible when tank mixed with many existing seed treatment fungicides and are very convenient to mix with flowable formulations such as Apron Maxx® RTA.

Development of a field inoculation method for Pythium blight of snap beans useful for field efficacy trials
L. HARRISON (1), S. L. Rideout (1)
(1) Virginia Tech, Painter, VA, U.S.A.
Phytopathology 100:S48

Pythium blight has become a severe disease threatening snap beans production in important growing areas, such as the Eastern Shore of Virginia (ESV). However, no effective in-season foliar fungicides currently have a Section 3 Federal label for control of Pythium blight. Labeling of fungicides for control is hindered by the difficulty associated with conducting trials with the pathogen(s), which occurs sporadically and clustered in the field. Different inoculum substrates and concentrations were evaluated in order to develop an inoculation technique that produces more uniform disease in field trials. Over 3 summers, substrates inoculated with an ESV *Pythium aphanidermatum* isolate were evaluated in the field, including sterilized soil/oatmeal (2% by weight), vermiculite/V8 juice (5:3 weight to volume), and long grain rice/water (5:3.6 weight to volume). Each inoculum substrate was applied at rates of 0, 2,500, 5,000, and 10,000 cfu at plant flower bloom. Disease incidence was recorded as percentage of diseased 0.3 m segments (foot rows). The V8/vermiculite inoculum substrate (5,000 cfu) consistently caused ~50% disease in each field trial. This treatment was used to provide disease pressure in a Pythium blight fungicide efficacy trial in 2009. Results indicated that azoxystrobin, cyazofamid, pyraclostrobin, and materials in the phosphonate class were most effective at reducing disease incidence and improving marketable yields when compared to the non-treated control. Field efficacy trials will be repeated in 2010.

Managing Rhizoctonia root and crown rot in Nebraska utilizing azoxystrobin applications based on soil temperature measurements
R. M. HARVESON (1)
(1) University of Nebraska, Scottsbluff, NE, U.S.A.
Phytopathology 100:S48

Rhizoctonia root and crown rot, caused by *Rhizoctonia solani*, is the most widespread, consistently damaging sugar beet disease in Nebraska, and causes both a seedling disease and two different phases of root rot later in season. These two phases include a crown rot, and a tip rot of the tap root beneath the soil surface. Because of the diversity of pathogen forms observed, making fungicide recommendations based on plant growth stage or chronological time of the season is difficult and impractical. Therefore, a study was begun in 2009 with the purpose of evaluating optimal timing for making fungicide applications based on measurement of soil temperature. Spray treatments were applied based when 10 cm soil temperatures averaged 15°, 18°, 21°, and 24°C for 3 sequential days, with two additional treatments consisting of an untreated check and spraying at symptom expression. Data collected included multiple disease counts, disease severity ratings assigned at harvest, and sucrose and root yield determinations. Sugar yields and numbers of diseased plants were significantly improved with the use of azoxystrobin when soil temperatures reached 15°, 18° and 21°C, compared to controls and spraying after symptom development. Therefore the general concept appears to work adequately for Nebraska conditions; however improvements may still be realized with some further modifications.

A survey to determine predominant diseases found in Nebraska sunflower production
R. M. HARVESON (1)
(1) University of Nebraska, Scottsbluff, NE, U.S.A.
Phytopathology 100:S48

Sunflower is a well-adapted crop for the Central High Plains and can be successfully cultivated in both dry-land and irrigated areas. It additionally fits well in many production systems as an alternative crop in dry-land wheat rotations. Sunflowers are also being increasingly used to lengthen the traditional irrigated rotations of dry beans, corn and sugar beets. As would be expected, the increase in acreage also increases the potential for disease problems to occur. Thus it would be important to develop a knowledge base for the prominent diseases found in the state. Thus a comprehensive disease survey was conducted in Nebraska production fields during the 2009 season. The survey consisted of 30 different fields, including 20 that were irrigated and 10 that were non-irrigated. Twenty-five of the fields were surveyed at least twice to correspond with different plant growth stages. Rust was the most commonly found, widespread disease throughout Nebraska production fields in 2009. Other familiar, expected diseases were identified including white mold, and Rhizopus head rot, but not at high levels. Several new and/or unknown diseases were additionally found including Verticillium wilt, apical chlorosis, downy mildew, several distinct leaf spots, and numerous root and stem/stalk rots.

Thousand cankers disease of walnut: Status in California
J. K. HASEY (1), R. M. Bostock (2), T. J. Michailides (3)
(1) University of California, Yuba City, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.; (3) University of California, Parlier, CA, U.S.A.
Phytopathology 100:S48

Thousand cankers disease of eastern black walnut (*Juglans nigra*) and related *Juglans* spp. has emerged as a disease of significant concern in the western U.S. The disease is caused by a newly described fungal pathogen, with a proposed name of *Geosmithia morbida*, and is spread from attacks by the walnut twig beetle (WTB, *Pityophthorus juglandis*) with subsequent canker formation in the phloem. These cankers eventually coalesce to girdle the stems and branches. Trees usually die within three years of initial symptom development that include upper crown yellowing and dark bark staining. The thousand cankers disease was first confirmed in CA in June, 2008 in Yolo Co. Since then, the beetle-fungus complex has been confirmed in many counties distributed across the state on four black walnut species, English walnut, and/or seedling Paradox hybrid walnut rootstock. English walnut planted for commercial nut production does not appear to be a preferred host for the beetle. *Botryosphaeria* spp. that can cause cankers and limb dieback on English walnut have also been isolated from some branches infected with thousand cankers. WTB is a native bark beetle first collected in 1959 in Los Angeles Co., but its association with *Geosmithia* spp. in CA has only recently been documented. What is unclear is why thousand cankers disease has emerged on such a wide scale in California and the western U.S.

A multiplexed, probe-based quantitative PCR assay for DNA of *Phytophthora sojae*
J. S. HAUDENSHIELD (1), G. L. Hartman (1)
(1) USDA ARS, Urbana, IL, U.S.A.
Phytopathology 100:S48

Phytophthora sojae (Kaufm. & Gerd.) causes seed rot, pre- and post-emergence damping-off, and sometimes foliar blight in soybean (*Glycine max*). Crop loss may approach 100% with susceptible cultivars. We report here the development of a unique quantitative PCR assay specific to DNA of *P. sojae*, and a matching exogenous control, suitable for multiplexing with other similar pathogen assays. The primers (previously reported) and probe for this fluorogenic, 5'-exonuclease assay target the DNA sequences of a gypsy-like transposable retroelement present in *P. sojae.* The assay exhibited a limit of detection of under 34 fg total *P. sojae* DNA (0.5 genome) in serial dilutions, and suggested that up to 10 copies of the target retroelement were present per genome. Losses during DNA extraction effected a practical detection limit of four zoospores per sample. The assay positively detected DNA from 13 different *P. sojae* isolates pathogenic on soybean, and excluded from detection 17 other species of *Phytophthora*, as well as 13 fungal species pathogenic on soybean. The exogenous internal control target validated negative calls, and the assay was successfully multiplexed with two additional assays to simultaneously detect DNA from the fungus *Fusarium virguliforme* and the nematode *Heterodera glycines. P. sojae* DNA is readily extracted from infested soil, seed and plant debris, and may now be quantified by real-time PCR for diagnostic, forensic or research purposes.

Investigating altered triazole sensitivity in *Rhynchosporium secalis*

N. Hawkins (1), H. J. Cools (1), M. W. Shaw (2), H. Sierotzki (3), B. A. FRAAIJE (1)
(1) Rothamsted Research, Harpenden, UNITED KINGDOM; (2) University of Reading, Reading, UNITED KINGDOM; (3) Syngenta Crop Protection, Stein, SWITZERLAND
Phytopathology 100:S49

Rhynchosporium secalis is a fungal pathogen causing barley leaf blotch or scald. It is highly economically damaging, causing yield losses of up to 40% when untreated. Control strategies generally rely on a combination of more resistant barley varieties and fungicide use, and the triazoles are a major fungicide group for the control of this disease. However, a reduction in sensitivity to some triazole fungicides had been reported in the field. This study aimed to characterise the *in vitro* triazole sensitivity of *R. secalis* isolates, and to identify the molecular mechanisms associated with sensitivity differences. A fungicide sensitivity bioassay was developed for *R. secalis*. Sensitivity tests revealed ten- to 100-fold reductions in *in vitro* sensitivity to some triazoles over the last 10–15 years. *Rhynchosporium secalis* has two copies of the gene, *CYP51*, encoding the triazole target site. Investigations into the roles of these genes in triazole sensitivity will be presented.

Identification and application of the rice broad-spectrum blast resistance gene *Pigm*

Z. HE (1), Y. Deng (2)
(1) Shanghai Institutes for Biological Sciences, CAS, Shanghai, PRC PEOPLES REP OF CHINA; (2) Shanghai, PRC PEOPLES REP OF CHINA
Phytopathology 100:S49

Rice blast is one of the most destructive diseases of rice. Identification and utilization of broad-spectrum resistance genes has been the most effective and economical approach to control the disease. A native Chinese variety, GM4, was identified with broad-spectrum and durable resistance. Map-based cloning identified that the locus *Pigm* with 13 NBS-LRR members confers broad-spectrum blast resistance and had undergone duplication during the evolution of the resistance gene cluster. In the *Pigm* locus, *Pigm1* and *Pigm2* control whole stage resistance to rice blast including and leaf and neck blast on the seedling and mature rice stage, and *Pigm3* confers the resistance to neck blast that leads to large loss of grain yield. Genetic and transcriptional analysis suggested that broad-spectrum resistance might be attributed to the different expression pattern of three *R* genes pyramided in the *Pigm* locus. Meanwhile we have succeeded in developing elite hybrid rice lines with broad-spectrum blast resistance using molecular markers-assisted selection for *Pigm*, indicating good potential of the gene in rice breeding.

Evaluation of leaf removal timing and method and gibberellic acid for grape bunch rot management

B. HED (1), H. K. Ngugi (2), J. W. Travis (2)
(1) Lake Erie Regional Grape Res & Ext Ctr, North East, PA, U.S.A.; (2) Penn State Fruit Research and Extension Center, Biglerville, PA, U.S.A.
Phytopathology 100:S49

Bunch rot caused by *Botrytis cinerea* is an important disease of wine grapes worldwide. Bunch rot development is strongly influenced by the compactness of clusters, which can be modified to reduce the disease. For 3 seasons, we evaluated cluster zone leaf removal timing (trace bloom, 2–3 weeks post bloom, or veraison) and method (by hand or Gallagher leaf blower) and bloom gibberellin sprays (5 or 10 ppm), for effects on cluster compactness and Botrytis bunch rot development on *Vitis vinifera* 'Chardonnay' grapevines. All treatments including the check received two *Botrytis* specific fungicides; cyprodinil at pre-close and fenhexamid at veraison. Botrytis bunch rot decreased the earlier the leaf removal was performed. Compared to the check, leaf removal at trace bloom significantly reduced ($P < 0.05$) bunch rot incidence in every season (by an average of 80 percent), and reduced cluster compactness and bunch rot severity in 2 of 3 seasons. Leaf removal at trace bloom was as effective at reducing bunch rot as 2 additional fungicides (cyprodinil at bloom and fenhexamid at pre-harvest), suggesting potential for reducing fungicide use. There was no significant effect of leaf removal method on bunch rot. Gibberellin reduced bunch rot by an average of 33 to 35 percent over the check, but the reductions were significant only at 5 ppm in 1 of 3 seasons. Gibberellin at 10 ppm significantly reduced cluster compactness in 2 of 3 seasons, when compared to the check.

Management of bunch rot of Vignoles and Chardonnay grapes with gibberellic acid

B. HED (1), H. K. Ngugi (2), J. W. Travis (2)
(1) Lake Erie Regional Grape Res & Ext Ctr, North East, PA, U.S.A.; (2) Penn State Fruit Research and Extension Center, Biglerville, PA, U.S.A.
Phytopathology 100:S49

Harvest bunch rot of wine grapes caused by *Botrytis cinerea* is a perennial problem in the eastern U.S. that can severely impact wine quality. Previously, we documented a strong relationship between cluster compactness and the incidence and severity of bunch rot on Vignoles grapes. The goal of this study was to investigate the effectiveness of gibberellic acid (GA) applications to loosen clusters as a means for controlling bunch rot on Vignoles and Chardonnay grapes. On Chardonnay, GA applications at 10 or 25 ppm significantly ($0.0422 \leq P \leq 0.0062$) reduced cluster compactness in a 3-year study with bloom applications being more effective than pre-bloom applications. On Vignoles, all bloom applications and the 25 ppm pre-bloom treatments reduced compactness in every year. Depending on the year, GA applications had mixed effects on the incidence and severity of bunch rot on Chardonnay. On Vignoles, combining GA treatments with two fungicide applications resulted in better control of harvest rots than the use of two additional fungicides in 2006 and 2008. Cluster compactness accounted for 52.7% of the variation in the incidence of bunch rot on Chardonnay, between 77.2 and 89.4% on Vignoles, and was negatively correlated ($0.731 \leq r \leq 0.857$; $P < 0.0001$) with percent spray coverage on berries. In spite of repeated treatment of the same vines for four consecutive years, no negative effects on yield from GA treatments were noted on Vignoles in three of the four years.

Ethylene biosynthesis and its effect on rice resistance to fungal infection

E. E. HELLIWELL (1), Q. Wang (2), Y. Yang (2)
(1) Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, State College, PA, U.S.A.; (2) Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S49

Recent evidences suggest that ethylene (ET) may play a positive role in host resistance to *Magnaporthe oryzae*, the causal agent of rice blast disease. In the ET biosynthetic pathway, the rate-limiting step is the conversion of S-adenosylmethionine (AdoMet) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS). Among the six *ACS* genes in rice, *OsACS1* and *OsACS2* are induced by *M. oryzae* within 24 hours of inoculation. In this study, we have taken the transgenic approach to further determine the role of ET biosynthesis in rice resistance to fungal infection. Using the RNA interference (RNAi) method, transgenic rice lines were generated with suppressed expression of single (*OsACS2*) and double (*OsACS1+2*) ACS genes. These RNAi lines have much lower levels of ethylene production as compared to nontransformed control lines. In addition, transgenic rice lines with inducible overexpression of *OsACS2* and increased levels of ET production were generated by placing the transgene under the control of a strong pathogen-inducible promoter. Currently, both ET-deficient and ET-overproducing lines are being evaluated for altered defense gene expression and host resistance to rice blast and sheath blight (*Rhizoctonia solani*). These transgenic lines will be valuable tools in elucidating the importance of ethylene biosynthesis in rice disease resistance.

Effect of an at tassel fungicide application on *Aspergillus* and *Fusarium* spp. infestations in harvested field corn in Mississippi

A. Henn (1), T. W. ALLEN (2), D. M. Ingram (3)
(1) Mississippi State University, Starkville, MS, U.S.A.; (2) Mississippi State University, Stoneville, MS, U.S.A.; (3) Mississippi State University, Raymon, MS, U.S.A.
Phytopathology 100:S49

Currently, fungicide use in field corn (Zea mays) to prevent diseases caused by *Aspergillus flavus* and *Fusarium* spp. relies on seed treatments. The recent shift in Mississippi production hectares from cotton to corn has been associated with increased marketing of strobilurin fungicides for numerous non-disease related issues and aflatoxin reduction. This marketing strategy occurred in response to a reported "plant health" benefit from a carefully timed, tassel (VT) fungicide application. However, unless an application reduced the levels of toxin producing fungi then the resultant toxin would likely still occur in the corn plant. As part of a larger project conducted from 2007 to 2009 to determine yield response to a VT strobilurin application, the presence of *Aspergillus*, *Fusarium* and other fungi were quantified from harvested corn. Large plot trials were conducted on producers' fields in the Mississippi Delta and small plot trials on experiment stations. Fungicide applications were made at VT using azoxystrobin + propiconazole (as Quilt), propiconazole (as Propimax), and pyraclostrobin (as Headline) along with an untreated control by airplane (n = 15) or by ground (n = 6). Trial locations contained at least four replicates. Propiconazole and pyraclostrobin significantly reduced *Aspergillus* and *Fusarium* colony numbers compared to the untreated. However, *Aspergillus* colony numbers were significantly increased with an azoxystrobin + propiconazole treatment.

Epidemiology of potato zebra chip in the Texas Panhandle
D. C. HENNE (1), F. Workneh (1), C. Rush (1)
(1) Texas AgriLife Research, Bushland, TX, U.S.A.
Phytopathology 100:S50

"Zebra Chip" (ZC), an emerging disease of potato in the U.S., is caused by the phloem-limited fastidious bacterial endosymbiont *Candidatus* Liberibacter solanacearum (CLs), which is vectored by the potato psyllid, *Bactericera cockerelli*. ZC leads to early plant death and causes internal tuber necrosis that renders them unmarketable. Although first observed in south Texas as early as 2000, no information was available concerning spatial and temporal incidence of ZC in potato fields. Therefore, to assess the incidence of ZC, studies were performed from 2007–2009 in potato fields at two commercial production areas in the Texas Panhandle. It was determined that, under field conditions, ZC-affected plants typically do not express symptoms of the disease until after flowering (although the vector is present much earlier), whereupon symptoms increase monotonically over several weeks to a plateau. Spatial incidence of ZC-affected plants generally follows a negative binomial distribution, with groups of diseased plants occurring close together. In most cases, incidence of ZC across potato fields yielded no discernable pattern. However, a regularly repeating pattern of disease incidence was present across at least one field. Incidence of ZC in potato fields did not correlate with psyllid abundance, or percentage of potato psyllids testing positive for CLs.

The impact of a strobilurin fungicide and other pesticides on soybean yield and yield components
R. HENRY (1), K. Wise (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S50

The use of strobilurin fungicides has increased in recent years in the United States. These fungicides reportedly improve plant health as well as control foliar diseases, and fungicide applications have been touted as a means to increase yield in many field crops. The objective of this study is to determine the impact of a strobilurin fungicide on soybean disease control, yield, and yield components, alone and in combination with other pesticides, in Indiana. Field research trials were established in Wanatah, West Lafayette, and Butlerville, Indiana in 2009. Foliar treatments consisted of glyphosate, manganese, pyraclostrobin, and a lambda-cyhalothrin insecticide, applied alone or in various combinations to two glyphosate-resistant soybean varieties. Control treatments were included. Disease incidence and insect populations were low throughout the season at all three locations. Fungicide treatment did not significantly ($P < 0.05$) increase yield of the newest class of glyphosate-resistant soybean at any location. Fungicide did have a significant effect on yield of the conventional glyphosate-resistant soybean at the West Lafayette and Butlerville locations. Inclusion of any other pesticide or micronutrient with a fungicide did not significantly improve the yield of either variety. The results of this study demonstrate the variability in the yield response due to a strobilurin fungicide application.

Evaluation of winter wheat cultivars for resistance to Fusarium head blight and deoxynivalenol
J. HERNANDEZ NOPSA (1), S. N. Wegulo (1)
(1) University of Nebraska, Lincoln, NE, U.S.A.
Phytopathology 100:S50

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a destructive disease of wheat. *F. graminearum* also produces the mycotoxin deoxynivalenol (DON) which contaminates grain. One strategy for managing FHB is to plant resistant cultivars. Field trials were conducted in Nebraska, U.S.A. in 2008 at Mead and in 2009 at Paxton to evaluate winter wheat cultivars for resistance to FHB and DON. Cultivars were arranged in a randomized complete block design with 3 or 4 replications. FHB index (percent) and DON (ppm) were measured. DON was measured in grain from symptomatic heads (DONtag) and in grain bulked from each plot (DONplot). Cultivars differed in FHB index at Paxton (20 cultivars, $P < 0.0001$) and Mead (12 cultivars $P < 0.0001$). Cultivars differed in DONtag at Mead ($P = 0.0015$), but not at Paxton ($P = 0.1249$). DONplot differed ($P < 0.0001$) among cultivars at Mead, but not at Paxton ($P = 0.1330$). DONtag averaged across cultivars was higher ($P < 0.0001$) than DONplot at both locations. At Paxton, FHB index ranged from 4 (Goodstreak) to 60 (Overley). DONtag ranged from 4.7 (Art) to 12.6 (Hatcher). DONplot ranged from 0.2 (Overland) to 4.3 (Postrock). At Mead, FHB index ranged from 13 (Harry) to 64 (Overley). DONtag ranged from 5 (2137) to 19 (Overley). DONplot ranged from 4 (Hondo) to 10 (Harry).

Analysis of viral DNA accumulation in pepper plants with two different strains and chimeras of *Pepper golden mosaic virus*
C. HERNANDEZ-ZEPEDA (1), A. M. Idris (1), J. K. Brown (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S50

Two strains of the bipartite begomovirus *Pepper golden mosaic virus* (PepGMV) cause different symptom phenotypes in pepper and tomato plants. The mosaic strain, PepGMV-Mo, causes severe, systemic yellow mosaic symptoms in pepper and mild symptoms in tomato, whereas, the distortion strain, PepGMV-Di, causes leaf distortion symptoms on inoculated leaves subsequent recovery in developing leaves, whereas it causes severe symptoms in tomato. We have mapped the 'remission' phenotype of the Di strain to a defective upstream non-coding (putative promoter) region of the PepGMV-Di cell-to-cell movement protein (BL1). Chimeric viruses were previously obtained by exchanging the BL1 promoter between the Mo and Di strains. We inoculated pepper (*Capsicum annum* var. Anaheim) plants with the wild type PepGMV-Mo and PepGMV-Di and with 'promoter chimeric' viruses. Plants were grown under environmentally controlled conditions for 21 days. Total DNA was isolated from pooled leaves (1 to 6) above the point of inoculation. Radioactive labeled viral DNA was used as probe to assess viral DNA accumulation. Results indicated there was no significant detectable decrease in viral DNA levels in the symptomatic, compared to recovered, leaves. This supports the hypothesis that the recovery DI phenotype is not due to reduced viral replication in systemically infected leaves, but rather to cell-to-cell and (likely) systemic movement owing to aberrant BL1 expression by a possibly defective promoter.

MeloCon WG® and SoilGard 12G® used in a program as a methyl bromide alternative to control nematodes and soil borne diseases in vegetable production
H. HIGHLAND (1)
(1) Certis USA, Nokomis, FL, U.S.A.
Phytopathology 100:S50

In reaction to the Montreal Accord of 2007 on restricting ozone depleting gases, effective and safe alternative treatments are being investigated, labeled and used in commercial production. The loss of fumigants is especially deleterious to the production of fresh market vegetables. In the southeastern U.S., soil borne diseases and nematodes can be of particular concern. A program of MeloCon® WG and SoilGard® 12 G, marketed by Certis USA, have been shown to be very effective when used alone or in combination to control nematodes and soil pathogens in field trials in the U.S. MeloCon® WG is a naturally occurring and beneficial soil fungus (*Paecilomyces lilacinus* strain 251) that controls a wide range of plant parasitic nematodes. MeloCon® WG has been shown in replicated field trials to control both southern root knot nematodes (*Meloidogyne incognita*) and stubby root nematodes (*Trichodorus* spp. and *Paratrichodorus* spp.), as well as many others. SoilGard® 12G is also a naturally occurring and beneficial soil fungus (*Gliocladium* (Trichoderma) *virens* strain GL-21) that controls a wide range of soil borne pathogens, including southern blight (*Sclerotium rolfsii*), *Fusarium* crown rot, and pepper blight (*Phytophthora capsici*). Replicated field trials using various fresh market vegetables with these products in conjunction with soil applied herbicides resulted in improved plant growth, increased survival, and increased yields, similar to methyl bromide and other chemical standards.

Characterization of the cytochrome b gene from three stone fruit infecting *Monilinia* species
J. HILY (1), S. Singer (1), K. Cox (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY, U.S.A.
Phytopathology 100:S50

Single amino acid substitutions in the cytochrome b (cyt b) protein are known to be responsible for resistance to Qo inhibitor (QoI) fungicides in many plant pathogenic fungi. In many instances this results from specific point mutation in the cyt b gene, leading to the replacement of glycine with alanine at position 143 within the protein (G143A). To date, little is known about the structure cyt b gene and its relation to QoI sensitivity in Monilinia species. The goal of this study was to clone and characterize the cyt b gene of three agriculturally important Monilinia species that cause brown rot of stone fruit: Monilinia fructicola, M. fructigena and M. laxa. Full-length genes were cloned from genomic DNA, while mRNA sequences were cloned from cDNA, and were compared to previously determined cyt b sequences from other fungal pathogens. The three Monilinia species were nearly identical at the protein level, but differed in the number and size of introns. *M. laxa* appears to have three amino acid differences in the Qo regions compared to *M. fructicola*, which may explain the differences in QoI sensitivity between the two species in regional populations. Finally, we sought to improve the available molecular detection methods for Monilinia spp. We developed a single set of cyt b-specific PCR primers that amplify differentially sized fragments allowing discrimination of the three Monilinia species commonly responsible for brown rot in North America and Europe.

Efficacy of *Streptomyces lydicus* and cover crops for management of Fusarium wilt of watermelon

J. HIMMELSTEIN (1), K. Everts (2), J. Maul (3)
(1) University of Maryland, College Park, MD, U.S.A.; (2) University of Maryland and University of Delaware, College Park, MD, U.S.A.; (3) Sustainable Agriculture Systems Lab. USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S51

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON) is a re-emerging threat to watermelon production in the eastern U.S. due in part to production changes such as the increase in triploid watermelon acreage. New management practices are necessary because triploid cultivars have little resistance to Fusarium wilt. Use of cover crops and biocontrol soil inoculations are potential ways farmers can improve the sustainability of their production systems. Efficacy of the biofungicide Actinovate (Natural Industries, Inc.), which has an active ingredient of *Streptomyces lydicus*, used alone and in combination with a tilled cover crop was evaluated in two locations in Maryland. The cover crops *Vicia villosa* (hairy vetch), *Trifolium incarnatum* (crimson clover), and *Secale cereale* (rye) were grown, rolled, and incorporated as a green manure into the soil prior to planting watermelon. FON was inoculated at the base of each seedling three days after transplanting at the Salisbury location and five days after transplanting in Beltsville. Actinovate was first applied to watermelon seedlings two weeks before transplanting and again six days after the FON inoculation. No visible wilt symptoms were observed at either location. However Actinovate significantly increased marketable fruit yield in plots inoculated with FON compared to inoculated plots with no Actinovate, or plots with no inoculation. Evaluation of Actinovate in greenhouse trials is underway.

Derivation and validation of a model to predict selection for fungicide resistance

P. H. HOBBELEN (1), N. D. Paveley (2), B. A. Fraaije (1), J. A. Lucas (1), F. Van den Bosch (1)
(1) Rothamsted Research, Harpenden, UNITED KINGDOM; (2) ADAS, High Mowthorpe, Duggleby, Malton, UNITED KINGDOM
Phytopathology 100:S51

The evaluation of fungicide resistance management strategies using mathematical models can help select options that are worth testing in the field. However, the usefulness of model predictions depends on their predictive power. To the best of our knowledge, none of the published fungicide resistance models have been validated. In this study, we aimed to derive and validate a mathematical model that predicts the selection for fungicide resistance in foliar pathogens of cereal crops. The model was validated against independent data from four field experiments quantifying selection for a mutation conferring resistance to a quinone outside inhibitor (QoI) fungicide in powdery mildew (Blumeria graminis f. sp. hordei) on spring barley (Hordeum vulgare). Fungicide treatments with azoxystrobin differed in the total applied dose and spray number. For each treatment, we calculated the observed selection ratio as the ratio of the frequency of the resistant strain at the end of the season and its frequency at the start of the season. On a scatter plot of log observed selection ratios on log predicted selection ratios, for all four experiments, the 45° line through the origin explained 89–90% of the variance in the observed selection ratios. We believe this is the first fungicide resistance model for plant pathogens to be rigorously validated. The model can now be used with some degree of confidence to identify potential anti-resistance treatment strategies.

The usefulness of mixtures of a single-site and a multi-site fungicide as resistance management strategy

P. H. HOBBELEN (1), N. D. Paveley (2), F. Van den Bosch (1)
(1) Rothamsted Research, Harpenden, UNITED KINGDOM; (2) ADAS, High Mowtorpe, Duggleby, Malton, UNITED KINGDOM
Phytopathology 100:S51

Single-site fungicides that attack one site in the genome of a fungal pathogen give sufficient disease control but are at-risk of resistance development, while the reverse is often true for multi-site fungicides. Aim of this study was to use mathematical modelling to predict whether mixtures of a single- and a multi-site fungicide delay the development of resistance against the single-site fungicide while maintaining a reasonable minimum level of disease control. We focused on a host-pathogen system consisting of winter wheat and Septoria tritici with pyraclostrobin as single-site and chlorothalonil as multi-site fungicide. The usefulness of the mixing of a single- and a multi-site fungicide as resistance management strategy was defined as the number of seasons that the disease-induced reduction of the green leaf area duration was less than 5%. We determined this measure for scenarios in which the dose rate 1) was constant for both chlorothalonil and pyraclostrobin, 2) was constant for chlorothalonil, but could increase for pyraclostrobin and 3) could increase for both fungicides but their ratio in the mixture was fixed. Doses could increase in between growing seasons as to maintain disease control. The effective life of pyraclostrobin was predicted to increase with increasing dose of chlorothalonil in the mixture from 3–4 years when applied alone to 9–12 years in mixtures with chlorothalonil close to or at the recommended dose.

Effect of chemical and biological control treatments against brown rot blossom blight in an organic sour cherry orchard

I. J. HOLB (1), M. Fazekas (2), F. Abonyi (2), P. Lakatos (2)
(1) University of Debrecen, Centre for Agricultural Sciences and Engineering, Debrecen, HUNGARY and Plant Protection Institute, Hungarian Academy of Sciences, Budapest, HUNGARY; (2) University of Debrecen, Centre for Agricultural Sciences and Engineering, Debrecen, HUNGARY
Phytopathology 100:S51

In a three-year study, the effect of lime sulfur, copper hydroxide, potassium carbonate and *Aureobasidium pullulans* treatments was evaluated against brown rot blossom blight in a Hungarian organic sour cherry orchard. Treatments were applied at recommended dosages at three times (closed blossom, full bloom, petal fall) during bloom on cultivar Érdi Bőtermő. In all years, copper hydroxide and lime sulfur alone were most effective for blossom blight control. Both treatments were not as effective as the conventional standard (penconazole) and caused significantly more damage on spur-leaf clusters and blossom during wet weather conditions. The effect of treatments of *Aureobasidium pullulans* and potassium carbonate alone were significantly better against blossom blight than untreated control. These treatments caused no damage on spur-leaf clusters and blossom but their efficacies against the disease were significantly lower than treatments of copper hydroxide and lime sulfur. A reduced dosage of copper hydroxide in combination with *Aureobasidium pullulans* increased efficacy against blossom blight but also increased phytotoxicity compared to treatment of *Aureobasidium pullulans* alone. Either efficacy or phytotoxicity of this treatment combination was not significantly higher compared to treatments of copper hydroxide and lime sulfur alone.

To what extent can winter pruning intensity reduce apple powdery mildew in integrated and organic apple orchards?

I. J. HOLB (1), M. Fazekas (2), B. Balla (2), F. Abonyi (2)
(1) University of Debrecen, Centre for Agricultural Sciences and Engineering, Debrecen, HUNGARY and Plant Protection Institute, Hungarian Academy of Sciences, Budapest, HUNGARY; (2) University of Debrecen, Centre for Agricultural Sciences and Engineering, Debrecen, HUNGARY
Phytopathology 100:S51

The effectiveness of winter pruning intensity against apple powdery mildew was investigated on a susceptible (cv. Jonathan), a moderately susceptible (cv. Idared) and a lowly susceptible cultivar (cv. Mutsu) in organic and integrated apple orchards. The area under the disease progress curve for shoot and fruit incidences was calculated to evaluate three winter pruning treatments (unpruned, weakly pruned and strongly pruned) from 2007 until 2009. The lowly susceptible cultivar (cv. Mutsu) showed no significant effect of pruning treatments on apple powdery mildew in either orchard. In the organic orchard, both strong and weak pruning significantly decreased mildew incidence on shoot and fruit on the susceptible cultivar (cv. Jonathan) compared to unpruned ones. The effects of pruning treatments on mildew incidence were significant only on shoot of the moderately susceptible cultivar (cv. Idared), in all years in the organic orchard. In the integrated orchard, strong pruning treatment showed significant effect only on shoot of the susceptible and of the moderately susceptible cultivars (cvs. Jonathan and Idared) compared to unpruned ones. Our findings indicated that winter pruning intensity is an essential element of powdery mildew control on susceptible and moderately cultivars in organic apple growing.

Two new homothallic species of *Phytophthora* from irrigation reservoirs and natural waterways in Virginia

C. HONG (1), P. Richardson (1), S. Ghimire (1), W. Hao (1), P. Kong (1), G. Moorman (2), J. Lea-Cox (3), D. Ross (3)
(1) VPI & State University, Virginia Beach, VA, U.S.A.; (2) Penn State University, University Park, PA, U.S.A.; (3) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S51

Two distinct genotypes (L2 and A-2) were recovered from irrigation reservoirs and a stream in Virginia, U.S.A. Following molecular, morphological and physiological examinations, the 'L2' genotype was named *Phytophthora aquimorbida* and the 'A-2' designated *Phytophthora* taxon '*aquatilis*'. Both species are homothallic. *P. aquimorbida* is characterized by its noncaducous and nonpapillate sporangia, lateral and intercalary chlamydospores, catenulate and radiating hyphal swellings, and plerotic oospores formed in globose oogonia mostly in the absence of an antheridium. *P.* taxon '*aquatilis*' produces plerotic oospores in globose oogonia mostly with a diclinous, paragynous antheridium, and semi-papillate, caducous sporangia with variable pedicel lengths but it does not produce chlamydospores nor

hyphal swellings. Sequence analyses revealed that the closest relatives are *P. hydropathica*, *P. irrigata* and *P. parsiana* for *P. aquimorbida* and *P. multivesiculata* for *P.* taxon '*aquatilis*'. The optimum temperature for culture growth is 30 and 20°C for *P. aquimorbida* and *P.* taxon '*aquatilis*', respectively. Both *P. aquimorbida* and *P.* taxon '*aquatilis*' were pathogenic to rhododendron plants and caused root discoloration, pale leaves, wilting, tip necrosis and dieback. Their plant biosecurity risk is also discussed.

Biological control with *Xylella fastidiosa* strain EB92-1 for the prevention of Pierce's disease development in mature, producing grapevines

D. HOPKINS (1)
(1) University of Florida, Apopka, FL, U.S.A.
Phytopathology 100:S52

Pierce's disease (PD) of grapevine, caused by *Xylella fastidiosa*, limits the grape industry in much of the southern U.S. Injection of a benign strain (EB92-1) of *X. fastidiosa* into transplants has controlled PD in new plantings of *Vitis vinifera* cultivars. While strain EB92-1 has been shown to be effective in preventing PD in new grape plantings, there are mature vineyards that are rapidly being destroyed by PD. A treatment is needed to protect mature vines already in fruit production against PD. In vineyards in Georgia with moderate PD pressure (<30% new infections/yr) developing in the untreated vines, injection of EB92-1 by pin-pricking new branches resulted in significantly lower PD incidence in the cv. Mourvedre. Four years after treatment, PD incidence was 23% in the untreated vines and 8% in the EB92-1 treated vines. Injection of EB92-1 by pin-pricking in vineyards with chronic PD and abundant vectors did not reduce PD incidence (>30% per year) and does not appear to work in this high pressure situation. However, drill and syringe injection of the main trunk in these vineyards with chronic PD did effectively reduce PD incidence in the cvs. Mourvedre and Merlot. In North Carolina in 2009, drill and syringe injection of the main trunk was a more effective means of treating mature hybrid grapevines than pin-pricking. Drill and syringe injection of strain EB92-1 appears to have the potential to control PD in mature, producing vineyards.

BDM1* regulates virulence in *Fusarium graminearum

P. HOREVAJ (1), J. Xu (2), B. H. Bluhm (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Purdue Universtiy, West Lafayette, IN, U.S.A.
Phytopathology 100:S52

Fusarium graminearum causes head blight of wheat, as well as ear, kernel, and stalk rots of corn. During pathogenesis, it can produce numerous mycotoxins, including zearalenone and trichothecenes such as deoxynivalenol. Although the genes encoding the biosynthetic enzymes required for mycotoxin biosynthesis have been identified in the fungus, the molecular mechanisms underlying pathogenesis and mycotoxigenesis are not fully understood. In this study, an ortholog of *BDM1*, a virulence factor in the chestnut blight pathogen *Cryphonectria parasitica*, was disrupted in *F. graminearum* via split-marker homologous recombination. The phenotype of the disruption mutant was pleiotropic, including morphological abnormalities (e.g., the formation of knotted hyphae) and a substantial reduction in virulence on wheat heads. The functional characterization of *BDM1* in *F. graminearum* expands the current working model of how pathogenesis is regulated and suggests that orthologs of *BDM1* serve as virulence factors in taxonomically diverse fungal pathogens.

A virulence factor of phytoplasma inducing witches' broom and dwarfism symptoms

A. Hoshi (1), S. Kakizawa (1), Y. Ishii (1), N. Kojima (1), K. Sugawara (1), Y. Okano (1), K. Maejima (1), K. Oshima (1), S. NAMBA (1)
(1) Laboratory of Plant Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, JAPAN
Phytopathology 100:S52

Phytoplasmas are bacterial plant pathogens that can cause devastating yield losses in diverse crops worldwide. They reside inside of host cells, and are transmitted by insect vectors. Phytoplasma-infected plants show symptoms of witches' broom and dwarfism called Tengu-su disease in Japan. The molecular mechanisms of symptoms such as Tengu-su disease have been unclear. We have determined the complete genomic sequence of *Candidatus* Phytoplasma asteris, and revealed that the phytoplasma genome encodes very few metabolic functions, implying that phytoplasma is highly dependent on metabolic compounds from its hosts. We also revealed that Amp, a surface membrane protein of phytoplasma, formed a complex with insect microfilament proteins, and the formation of Amp-microfilament complex was observed only with the phytoplasma-transmissible insects, suggesting that this complex have a major role in determining the transmissibility of phytoplasmas. Here, we show that a secreted protein of phytoplasma, named TENGU, induces witches' broom and dwarfism symptoms. *Nicotiana benthamiana* and *Arabidopsis thaliana* plants expressing TENGU showed symptoms of witches' broom and dwarfism, which are typical of phytoplasma infection. Microarray analyses showed that auxin-responsive genes were significantly down-regulated in the *tengu*-transgenic plants. These results suggest that TENGU inhibits auxin-related pathways, thereby affecting plant development.

Genetic structure of *Phytophthora infestans* population in eastern North America, 2002–2009

C. HU (1), F. G. Perez (2), R. Donahoo (3), A. McLeod (4), K. L. Myers (4), K. L. Ivors (5), P. D. Roberts (3), W. E. Fry (4), K. L. Deah (2), J. B. Ristaino (6)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) USDA-ARS/PSI-Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville, MD, U.S.A.; (3) IFAS-SWFREC, University of Florida, Immokalee, FL, U.S.A.; (4) Dept. of Plant Pathology, Cornell University, Ithaca, NY, U.S.A.; (5) MHCREC, NC State University, Mills River, NC, U.S.A.; (6) Dept. of Plant Pathology, NC State University, Raleigh, NC, U.S.A.
Phytopathology 100:S52

Late blight caused by *Phytophthora infestans*, reemerged in the U.S. in 2009, and was the worst in modern history due to a "perfect storm" of widespread inoculum distribution and conducive weather. Two new genotypes named US-20 and US-21 were found on tomato in FL and NC between 2002 and 2007. More than 80 isolates of *P. infestans* were collected from 11 states and Canada. The US-8 genotype was found in potato crops in 5 states. In addition, three new genotypes, US-22, US-23 and US-24, were recovered from both potato and tomato. US-22 (A2; Gpi: 100/122) was widespread on tomato transplants sold in home garden centers that later spread to nearby commercial tomato or potato fields. US-23 (A1; Gpi: 100/100) was found in four states on both tomato and potato. US-24 (A1; Gpi: 100/100/111) was found only on potato in ND. *P. infestans* populations between tomato and potato were genetically differentiated. Migration analysis suggested that gene flow occurred from tomato to potato in the eastern U.S. populations. Isolates from a home garden in TN, a single site in NY in 2007, and FL in 2008 were also US-22. Coalescent analysis documented that the 2009 populations were derived from the 2007 US-22 population. The data indicate that the US-22 existed before the epidemics of 2009. Genotype diversity was greatest in PA and both mating types occurred there. The severe late blight epidemics of 2009 underscore the need for an improved web-based tracking and monitoring system for the pathogen in the U.S.

Development and use of an efficient and temperature-insensitive virus-induced gene silencing system in *Nicotiana tabacum*

C. HUANG (1), X. Zhou (1)
(1) Institute of Biotechnology, Zhejiang University, Hangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S52

Virus-induced gene silencing (VIGS) is a recently developed technique for characterizing the function of plant genes by gene transcript suppression and is increasingly used to generate transient loss-of-function assays. Recently we described that the geminivirus satellite vectors (2mDNA1 and DNAmβ) can trigger efficient gene silencing in many permissive host plants excluding *Nicotiana tabacum* when co-inoculated with the helper virus Tomato yellow leaf curl China virus. Here we report that the 2mDNA1 vector can induce efficient gene silencing in *N. tabacum* with Tobacco curly shoot virus. We have successfully silenced the *β-glucuronidase* (GUS) gene in the GUS transgenic *N. tabacum* plants and *sulphur desaturase* (Su) gene in the five different *N. tabacum* cultivars. These pronounced and severe knockout phenotypes are persistent and ubiquitous. Once initiated in seedlings, the silencing phenotype lasted for the entire life span of the plants and silencing could be induced in a variety of tissues and organs including leaf, shoot, stem, root and flower, and could be achieved at any growth stage. This system works well between 18–32°C. We also silenced *NtEDS1* gene and demonstrated that *NtEDS1* is essential for *N* gene mediated resistance against Tobacco mosaic virus (TMV) in *N. tabacum*. The above results indicate that this system has great potential as a versatile VIGS system for routine functional analysis of genes in *N. tabacum*.

Suppression of Fusarium crown and root rot in tomato by silicon

C. Huang (1), P. ROBERTS (2), L. Datnoff (3)
(1) University of Florida, GCREC, Wimauma, FL, U.S.A.; (2) University of Florida, SWFREC, Immokalee, FL, U.S.A.; (3) Department of Plant Pathology & Crop Physiology, LSU, Baton Rouge, LA, U.S.A.
Phytopathology 100:S52

Fusarium oxysporum f. sp. *radicis-lycopersici*, the causal agent of Fusarium crown and root rot (FCRR), is an important soilborne pathogen of tomato in south Florida. Although fumigation and host resistance may reduce the impact of this disease, other alternative management strategies are needed. Since silicon (Si) has been shown to reduce a number of fungal plant diseases, the purpose of this study was to determine if this element could suppress the

development of FCRR. Si significantly reduced the severity of FCRR on the stem of tomato 4-weeks after inoculation. Further analysis of disease progress suggested that the decrease in FCRR disease severity by Si amendment probably resulted from delaying the initial infection in roots and the movement of the pathogen from roots to stems. Si contents of roots and shoots were significantly higher in tomato plants with Si in comparison to those treatments without Si. Moreover, the increase in the Si content of roots was significantly correlated with the reduction of disease severity of root, crown, and stem, indicating a silicon-induced resistance and/or reduction of fungal colonization. Si treatments probably limited the basipetal spread of FORL from infected roots to stems. Although laboratory experiments including this study have shown that Si can alleviate biotic and abiotic stresses in tomato, further research in applying Si fertilizers for field-grown tomatoes needs to be conducted to further elucidate the effects of Si.

Microarray analysis identified *Puccinia striiformis* f. sp. *tritici* genes involved in infection and sporulation

X. Huang (1), X. CHEN (2), T. Coram (2), M. Wang (3), Z. Kang (1)
(1) Northwest A&F University, Yangling, Shaanxi, PRC PEOPLES REP OF CHINA; (2) USDA ARS, Pullman, WA, U.S.A.; (3) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S53

Puccinia striiformis f. sp. *tritici* (*Pst*) causes stripe rust, one of the most important diseases of wheat worldwide. To identify *Pst* genes involved in infection and sporulation, a custom oligonucleotide Genechip was made using sequences of 442 genes selected from *Pst* cDNA libraries. Microarray analysis was conducted by hybridizing the Genechip with cDNA from urediniospores (Ure), germinated Ure, and *Pst*-infected and mock-inoculated leaves sampled at 12 h, 24 h, 48 h, 7 d, and 14 d after inoculation. The time course study identified 55 genes that were differentially induced during the infection process. Nine of the genes were induced in both Ure and the sporulation stage of infection. Genes in this group mostly have functions in carbohydrate and lipid metabolism. Six genes, including a mitochondrial ATP synthase, a copper-induced metallothionein, a differentiation-related protein Infp, and a hypothetical cell wall mannoprotein, were induced during the early infection process and therefore, likely involved in pathogenicity. Four genes, including an exo-1,3-Beta-glucanase, a chitin deacetylase, and a meiotic recombination-related protein, were induced in both early infection and sporulation stages. Thirty five genes were induced in the sporulation stage, but not in Ure, indicating that they are involved in reproduction. One gene from the haustorial library with unknown function was only induced in infected leaves.

Identification and characterization of the causal agent of a new viral disease on sweet pepper in Taiwan

C. HUANG (1), Y. Zheng (1), Y. Cheng (2), C. Chen (1), F. Jan (1)
(1) National Chung Hsing University, Taichung, TAIWAN; (2) Division of Plant Pathology, Agricultural Research Institute, Taichung, TAIWAN
Phytopathology 100:S53

A virus culture TwPep1 was isolated from leaves of a sweet pepper plant (*Capsicum annuum* cv. Andalus) showing viral disease-like symptoms of chlorosis, chlorotic spots in the fields in central Taiwan in July, 2009. A partial L gene of tospovirus was amplified from the total RNA isolated from TwPep1-infected plant by RT-PCR with a degenerate primer pair for tospoviruses. The 819-nt L RNA conserved region of TwPep1 shared 94.4–97.7% nucleotide identity with those of *Tomato spotted wilt virus* (TSWV) available in GenBank. A 29 KDa protein of TwPep1 reacted positively with antiserum against the N protein of TSWV using a western blot. The nucleocapsid (NP) gene of the TwPep1 was amplified by RT-PCR using primers for the N gene of TSWV. The 777-bp N gene of TwPep1 shared 98.3–99.1% nucleotide and 98.5–99.6% amino acid identity with that of 21 TSWV isolates with full-length sequences of S RNA available in GenBank. The TwPep1 isolate was back-inoculated onto sweet pepper plants for pathogenicity test. The inoculated plants showed symptoms of chlorosis and chlorotic spots which were similar to that observed in the field. Electron microscopic examination showed about 80–100 nm in diameter in ultrathin sections of TwPep1-infected sweet pepper. Taken together, it is concluded that the causal agent of the new sweet pepper disease in Taiwan is indeed an isolate of TSWV. This is also the first demonstration of isolation and characterization of TSWV in Taiwan.

Molecular characterization of the *Enterobacter cloacae*-onion (*Allium cepa*) interaction and the search for pathogenicity determinants

J. L. Humann (1), S. Dossey (1), J. Peña (1), N. Peterson (1), A. A. Bates (1), R. Liesche (1), T. Peever (1), B. K. SCHROEDER (1)
(1) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S53

Enterobacter cloacae causes Enterobacter decay of onion bulbs in storage and is an emerging bacterial pathogen of onion (*Allium cepa*). *E. cloacae* is ubiquitous in nature and is an opportunistic pathogen of humans. A multilocus phylogeny demonstrated that strains of *E. cloacae* obtained from onion bulbs occupy a well-supported clade distinct from isolates of medical origin. Little is known about the *E. cloacae*-onion interaction and our investigations have demonstrated that *E. cloacae* can move through onion leaf tissue at a rate of approx. 2 cm per week. In addition, preliminary evidence suggests that *E. cloacae* biofilm mutants move faster *in planta* than wild-type strains. SEM studies have indicated that a matrix forms in the phloem of *E. cloacae* inoculated onion potentially reducing the size of the plant. Analysis of *E. cloacae* culture filtrates determined that a necrosis-inducing product is secreted into the medium that is both heat-instable and sensitive to proteinase K. In an effort to identify genes involved in the production of this necrosis-inducing product and pathogenicity, an onion slice assay was developed. This assay enabled the high-throughput screening of mini-Tn*5* mutants of *E. cloacae* and the identification of putative pathogenicity-minus mutants. These mutants are currently being characterized and genetic analysis of these *E. cloacae* mutants will be discussed.

Etiology of tomato yellow leaf curl disease complex in the Sultanate of Oman involves two helper begomoviruses, a betasatellite, and a DNA-2 satellite

A. M. Idris (1), J. K. BROWN (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S53

Rolling circle amplification was used to amplify, clone, and sequence the genomes of suspect begomoviruses and satellites from field grown tomato plants collected in 2005 in Oman. *Tomato yellow leaf curl virus* from Oman (TYLCV-OM), and an associated beta satellite have been reported previously by our group to be associated with symptomatic tomato plants. From the same samples, we have recently cloned a second, recombinant begomovirus that is sufficiently divergent from other begomoviruses to warrant its' classification as the distinct species, Tomato leaf curl Oman virus (ToLCOMV). Also cloned from the same sample was an alphasatellite-like molecule, referred to herein as TYLCDNA2 that is surprisingly closely related to another alphasatellite (AYVDNA2) from *Ageratum* in Singapore. The cloned ToLCOMV induced mild leaf curl symptoms in *Nicotiana benthamiana* and tomato, while TYLCV-OM infected plants developed more severe symptoms than those infected by TYLCV-Om. The addition of the betasatellite intensified the symptoms of both of the helper viruses. However, co-inoculation of plants with either of the helper viruses, plus the beta satellite and the TYLCDNA2 satellite, resulted in amelioration of symptom severity in both test plants. This is the first report of amelioration of disease symptoms in a natural and experimental host by a DNA-2 satellite in the presence of a helper virus and associated beta satellite.

Virulence and molecular characterization of *Verticillium* species from spinach

A. IGLESIAS-GARCIA (1), C. Feng (1), L. du Toit (2), K. Subbarao (3), J. Correll (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Washington State University Mount Vernon NWREC, Mount Vernon, WA, U.S.A.; (3) University of California, Davis, Salinas, CA, U.S.A.
Phytopathology 100:S53

Verticillium wilt, caused by *Verticillium dahliae*, is a widespread, economically important disease affecting >200 plant species. *V. dahliae* is a pathogen of spinach seed crops, in which symptoms develop only after the initiation of bolting (onset of reproductive growth). The objective of this study was to characterize molecular diversity, parasitic ability (root infection, vascular colonization), and host specificity among a diverse collection of *V. dahliae*, *V. tricorpus*, *Gibellulopsis nigrescens* (= *V. nigrescens*), *V. albo-atrum*, and *Lecanicillium fungicola* (= *V. fungicola*) isolates. Isolates of *Verticillium* species from spinach and several other hosts were evaluated for variability using the ITS region, mtDNA RFLPs, and putative species-specific markers. Pathogenicity tests in the greenhouse on spinach, tomato, and cotton plants with root-dip or root-drench inoculations revealed a wide range of host specificity and virulence among isolates. Isolates of *V. dahliae* that originated from spinach were pathogenic on spinach, whereas some isolates that originated from other hosts were not pathogenic on spinach. Isolates of *V. tricorpus* and *G. nigrescens* recovered from spinach seed, and isolates of *V. albo-atrum* and *L. fungicola* were not pathogenic on spinach. Together, these results indicate that *Verticillium* and related species associated with spinach display substantial variability in parasitic ability, virulence, and pathogenicity to spinach.

Species limits and evolution in *Verticillium*, a group of vascular wilt-pathogens of global importance

P. INDERBITZIN (1), R. M. Bostock (1), R. M. Davis (1), K. V. Subbarao (1)
(1) University of California, Davis, CA, U.S.A.
Phytopathology 100:S53

Verticillium is a diverse group of ascomycete pathogens of mushrooms, insects and plants. Here we focus on *Verticillium* sensu strictu (s.s.), a monophyletic group of plant pathogens closely related to *Glomerella* (anamorph *Colletotrichum*). Like many strains of *Fusarium oxysporum*, *Verticillium* s.s. causes vascular wilts 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 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 and evolution 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.

Flutriafol for control of cotton root rot, caused by *Phymatotrichopsis omnivora*

T. ISAKEIT (1), R. R. Minzenmayer (2), A. Abrameit (3)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) Texas AgriLIFE Extension Service, Ballinger, TX, U.S.A.; (3) Texas AgriLIFE Extension Service, Thrall, TX, U.S.A.
Phytopathology 100:S54

Cotton root rot (CRR), caused by the fungus *Phymatotrichopsis omnivora*, is a serious disease in many of the cotton production areas of Texas and other southwestern states. The objective of this study was to evaluate flutriafol at several rates for control of CRR in field experiments. The fungicide was applied prior to flowering, via drip irrigation or as a spray directed towards the lower stem. When applied via drip irrigation in a San Angelo trial, 0.125 lb a.i./A significantly ($P < 0.05$) reduced CRR incidence to 18%, in comparison to 52% incidence with the control. A yield (lint + seed) of 7405 lb/A was significantly ($P < 0.05$) greater with this rate, in comparison to the control yield, which was 5016 lb/A. Disease incidence was lower (33%, NS) with two applications each of 0.0625 lb a.i./A, separated by three weeks, but the yield of 6519 lb/A was significantly ($P < 0.05$) greater than the control. Rates of 0.0625, 0.125 and 0.25 lb a.i./A applied as a spray in 40 gpa to the stem significantly ($P < 0.05$) reduced CRR to 20%, 22%, and 12% incidence, respectively, in comparison with the control, 55%, in a trial in Williamson county. The disease incidences were also lower with the same rates of stem-directed sprays applied in San Angelo, 37%, 43%, and 32%, respectively, but these differences were not significantly ($P < 0.05$) less than the control, which was 57%. Flutriafol shows promise for economical control of CRR and further field experiments are underway to optimize effectiveness.

Involvement of chloroplast localized reactive oxygen species in promoting host and nonhost bacterial pathogens induced cell death

Y. ISHIGA (1), T. Ishiga (1), T. Wangdi (1), C. Ryu (1), K. S. Mysore (1), S. R. Uppalapati (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 100:S54

Coronatine (COR), a virulence factor produced by *Pseudomonas syringae* pv. *tomato* (*Pst* DC3000), is known to induce chlorosis during disease development. Recently we demonstrated that COR targets chloroplast to modulate reactive oxygen species (ROS) homeostasis in promoting disease-associated necrotic cell death. To further understand the role of COR in symptom development, we utilized *Nicotiana benthamiana* and virus-induced gene silencing (VIGS) as a forward genetics approach and identified several genes with altered responses to COR leading to necrotic cell death. One of the identified genes is chloroplast *Peroxiredoxin* (*Prx*). *Prx*-silenced *N. benthamiana* and tomato plants showed necrosis-like phenotype in response to COR. To investigate the involvement of chloroplast-localized Prx during the *Pst* DC3000-host interaction, we used *Arabidopsis prx* mutants. In pathogenicity assays, mutations in any of the five chloroplast *prx* genes did not resulted in altered disease symptom development. However, mutations in *NADPH-dependent thioredoxin reductase* (*NTRC*), an electron donor for Prxs in the NADPH-dependent thioredoxin (Trx) system resulted in accelerated *Pst* DC3000 disease-associated necrotic cell death in *Arabidopsis*. Furthermore, *ntrc* mutant showed accelerated cell death in response to nonhost pathogens, including *Pseudomonas syringae* pv. *tabaci*, pv. *glycinea*, and pv. *tomato* T1. These results suggest chloroplast ROS might play a key regulatory role in promoting cell death during both host and nonhost interactions.

A *Medicago truncatula* resistance to rust (*rer*) mutant displays enhanced resistance to *Phakopsora pachyrhizi* but not to necrotrophic fungal pathogens

Y. ISHIGA (1), S. Mittal (1), S. R. Uppalapati (1), V. Doraiswamy (1), H. Schultheiss (2), K. S. Mysore (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) BASF Plant Sci GmbH, Limburgerhof, GERMANY
Phytopathology 100:S54

Asian soybean rust caused by the fungus *Phakopsora pachyrhizi* is one of the most devastating foliar diseases affecting soybeans grown worldwide. Understanding the plant defense mechanisms and signaling pathways to *P. pachyrhizi* would assist in development of resistant plants. The most common form of plant defense mechanism against potential pathogens in nature is nonhost resistance. *Medicago truncatula* is a model plant species for legumes, and shows nonhost resistance response to *P. pachyrhizi*. To identify *M. truncatula* mutants with altered resistance to *P. pachyrhizi*, we have established a forward-genetic screen of *M. truncatula Tnt1* insertion lines. Screening of more than 1000 *Tnt1* lines identified several interesting mutant phenotypes. *Tnt1* insertion mutant of one of the mutant, *rer* (Resistance to rust), showed resistance to *P. pachyrhizi* by supporting less spore adhesion and germ-tube elongation. Further, *rer* mutant showed resistance to hemibiotrophic pathogen, *Colletotrichum trifolii*, but not to necrotrophic pathogens, *Phoma medicaginis* and *Sclerotinia sclerotiorum*. Interestingly, *rer* mutant has five leaves and shows less leaf surface hydrophobicity, suggesting that chemical or physical surface signals of *rer* mutant may play an important role in altered *P. pachyrhizi* spore adhesion and infection structure formation leading to enhanced resistance.

Pantocin A antibiotic produced by *Pantoea vagans* C9-1: Chemical and genetic characterization

C. A. ISHIMARU (1), T. Lansdell (2), J. Gross (3), J. Clardy (3), T. H. Smits (4), B. Duffy (4)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) Colorado State University, Fort Collins, CO, U.S.A.; (3) Harvard Medical School, Boston, MA, U.S.A.; (4) Agroscope Changins-Wädenswil ACW, Wädenswil, SWITZERLAND
Phytopathology 100:S54

Pantoea vagans C9-1 is one of the most effective and reliable biocontrol agents against fire blight, and has been commercialized as Blight Ban C9-1. Production of multiple antibiotics contributes to antagonism. Here we describe the genetics, chemical isolation, and structure of pantocin A (formerly reported as herbicolin O), the histidine sensitive antibiotic produced by *P. vagans* C9-1. Mutational analysis indicated that biosynthesis of pantocin A required *paaAB* and a sequence encoding the peptide precursor of pantocin A. Complete genome sequencing of *P. vagans* C9-1 identified the *paaABC* gene cluster encoding biosynthesis and autoresistance, located on a 28 kb chromosomal genomic island. PCR analysis indicated absence of *paaABC* in other *P. vagans* and presence in some *P. agglomerans* strains. The cluster was cloned in *E. coli* and purified antibiotic was isolated using improved methods for small peptides. The ^{1}H NMR spectra of the C9-1 antibiotic closely resembled those of pantocin A produced by *P. agglomerans*. Detailed analysis of the proton spin systems via a dqf-COSY NMR and HMQC NMR experiments showed that the chemical shift values and coupling constants of the protons in C9-1 herbicolin O correspond exactly to those of pantocin A. Based on these genetic and chemical analyses, herbicolin O and pantocin A are the same molecule.

Phenology of natural inoculation of apples by sooty blotch and flyspeck fungi in Iowa apple orchards

S. ISMAIL (1), J. C. Batzer (1), D. A. Mayfield (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S54

The sooty blotch and flyspeck (SBFS) complex is comprised of epiphytic fungi that cause dark blemishes on apples. In 2009, we investigated the timing of natural inoculation of apple fruit by SBFS species in six Iowa orchards. Five trees in each orchard received no fungicide sprays after first cover. Within 1 week after first cover, 'Golden Delicious' apples in each orchard were covered with Japanese fruit bags to exclude SBFS inoculum. Subsequently, five apples per tree were exposed for 2-week periods and then re-bagged for the remainder of the growing season, for a total of seven exposure periods. Controls included apples that were bagged for the entire season or exposed all season. At harvest, fruit bags were removed and apples were sorted by exposure period and stored at 4°C for 6 weeks. Colonies of SBFS fungi on each fruit were counted, then excised with the subtending peels and pressed between paper towels. During each of the first three exposure periods, about 70% of the apples developed colonies, with an average of 3.0 SBFS colonies per apple. During the seventh exposure period, 21% of apples were infested, with a mean of 1.3 colonies per apple.

Approximately 4% of apples that were bagged all season had 1 or 2 SBFS colonies located close to the stem end. Results of molecular-based identification of SBFS species will yield species-specific patterns for timing of apple inoculation.

Detecting resistance to QoI fungicides in *Alternaria solani* isolates collected from tomatoes in North Carolina

K. IVORS (1), L. Lacey (1), D. Milks (1), G. Olaya (2)
(1) North Carolina State University, Mills River, NC, U.S.A.; (2) Syngenta Crop Protection, Vero Beach, FL, U.S.A.
Phytopathology 100:S55

During the 2007 and 2008 growing seasons, some North Carolina (NC) growers observed unsatisfactory levels of early blight control after standard applications of QoI fungicides. *Alternaria solani* isolates were collected from four tomato fields in NC during the 2008 growing season. DNA extracts from 75 isolates were subjected to cytochrome b sequencing to determine if they possessed any of the mutations correlated with reduced sensitivity to QoI fungicides. Twenty-seven isolates with the F129L mutation (substitution of the amino acid phenylalanine for leucine at position 129 of the cytochrome b gene) were detected in 3 of the 4 fields. This F129L mutation can be produced by the 3 different single nucleotide polymorphisms (SNPs) TTA, CTC and TTG. Among these 27 isolates, the most common SNP was CTC followed by TTA; TTG was not detected. All 20 isolates collected from one of these commercial fields with a long history of consecutive tomato cropping possessed the F129L mutation with either the CTC or TTA SNP. We are currently conducting *in vivo* sensitivity tests on all isolates using the QoI fungicide azoxystrobin on Mountain Fresh tomato plants to combine results from both molecular and plant assays.

Diversity of *Phytophthora capsici* from vegetable crops in Georgia

K. JACKSON (1), J. Yin (1), A. Csinos (1), H. Scherm (2), P. Ji (1)
(1) University of Georgia, Tifton, GA, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S55

Phytophthora blight caused by *Phytophthora capsici* is a major concern in vegetable production in Georgia and other southeastern states. Studies were conducted to determine the diversity of *P. capsici* from vegetable crops in Georgia. Sporangia of the isolates ranged from 38.5 to 55.8 µm in length with length to width ratios ranging from 1.4 to 2.0. The diameters of oospores ranged from 24.8 to 30.4 µm, with no considerable differences among isolates from different hosts. All the isolates tested could grow at 35°C, but growth rates of the isolates differed at all the temperatures evaluated. Studies with susceptible and tolerant bell pepper cultivars under greenhouse conditions indicated that there were significant differences among the isolates in aggressiveness. The majority of the isolates were sensitive to 100 ppm of mefenoxam but insensitive to 100 ppm of cyazofamid, while all the isolates were sensitive to fluopicolide or mandipropamid. EC_{50} values in suppressing mycelial growth, zoospore germination, and sporangium production averaged 0.2, 2.7 and 1.7 ppm for fluopicolide and 0.02, 6.6 and 0.02 ppm for mandipropamid. Analysis of the variability of *P. capsici* isolates in Georgia using different molecular markers indicated that the isolates were genetically distinct. These results suggest that *P. capsici* populations infecting vegetable crops in Georgia are genetically diverse, which should be considered in developing resistant cultivars or other disease management programs.

***In planta* expression profiling reveals *Ralstonia solanacearum* physiology and the importance of sucrose metabolism during bacterial wilt of tomato**

J. M. JACOBS (1), F. Meng (1), L. Babujee (1), C. Allen (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S55

At early stages of disease development, cells of the bacterial wilt pathogen *Ralstonia solanacearum* (Rs) reach densities of 1e8-1e9 CFU/g stem and remain xylem-limited. Little is known about what compounds Rs metabolizes to colonize the low-nutrient xylem environment. A comparative microarray analysis of two ecologically distinct Rs strains, tropical GMI1000 (phylotype I) and temperate UW551 (phylotype II), gave a global overview of the cellular processes used during tomato pathogenesis. Most known virulence factors (e.g. cell wall degrading enzymes, exopolysaccharide, protein secretion) were highly expressed *in planta*. Several central primary metabolic pathways (such as sucrose, nitrate and myo-inositol metabolism) were induced in tomato stems. Notably, a sucrose-specific phosphoenolpyruvate phosphotransferase (PTS) metabolic cluster (*scrRABYK*) was highly expressed in both strains, suggesting Rs encounters sucrose in the xylem environment. Rs is the only known β-proteobacterium with a sucrose-specific PTS cluster. Mutants lacking ScrA (sucrose-6-phosphotransferase) could not use sucrose as sole carbon source. Following naturalistic soil-soak inoculation of tomato plants, *scrA* mutants were delayed in both virulence and host colonization when compared to wild-type. This functional genomic analysis suggests Rs depends on host sucrose to thrive in the host environment.

Comparative in planta microarray analysis modifies the regulatory model for the type three secretion system in *Ralstonia solanacearum*

J. M. JACOBS (1), F. Meng (1), L. Babujee (1), A. Milling (1), C. Allen (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S55

The regulatory mechanisms that govern virulence factor gene expression in the bacterial wilt pathogen *Ralstonia solanacearum* (Rs) are complex and have been primarily studied *in vitro*. We used transcriptome analysis to compare gene expression in tomato stem tissue and rich medium of two distinct Rs strains: GMI1000 (phylotype I) and UW551 (phylotype II). About 25% of the approximately 3500 shared orthologous genes were differentially expressed ($P < .05$) during early symptom development of tomato (~5e8 CFU/g stem) when compared to rich medium (6e8 CFU/g). Our *in planta* microarray data agreed with previous findings that the virulence factor exopolysaccharide is abundantly expressed during early symptom development at high cell densitites (>5e8 CFU/g stem). However, genes encoding the type three secretion system (T3SS) and effectors were also strongly expressed at high cell densities *in planta*, contrary to the model based on *in vitro* data. In culture the global virulence regulator PhcA represses the expression of T3SS regulator *hrpB* at high cell densities; the current model posits that T3SS is important early in disease but not later. In contrast, our *in planta* study showed that both EPS and T3SS genes were highly expressed even at later stages of disease development and thus at high cell densities. This suggests that the biological role of T3SS extends beyond early disease, and that *in vitro* regulatory studies can yield misleading results.

Influence of crop rotation on persistence of the atoxigenic strain *Aspergillus flavus* AF36 in Arizona

R. JAIME-GARCIA (1), P. J. Cotty (2)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) USDA ARS/University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S55

Aflatoxins are toxic and carcinogenic secondary metabolites produced by fungi of the genus *Aspergillus* and frequently contaminate cottonseed in Arizona. Several methods to contain aflatoxins have been devised. The use of atoxigenic strains of *A. flavus*, including strain AF36, to displace aflatoxin producers is a commercially proven technology. Previous work indicates that atoxigenic strain applications can benefit subsequent crops in treated fields and that applications have beneficial influences for several years. However, factors that influence atoxigenic strain persistence are not well documented. The current study sought to determine influences of cropping practices and rotation on persistence of AF36 in desert production areas of Arizona. Quantities of *A. flavus* in fields treated with the AF36 biocontrol were highest immediately after harvest, declining significantly once winter crops were planted. However, the percent of the *A. flavus* community composed of AF36 was not significantly affected. Structures of *A. flavus* communities were significantly affected by spring and summer crops. Fields cropped to wheat had significantly lower percentage of aflatoxin producer strains and highest of AF36 than fields planted to other crops. Results indicate that agronomic practices influence both the quantity and quality of *A. flavus* resident in fields and that practices might be optimized to maximize long-term displacement of aflatoxin producers by atoxigenic biocontrols.

Comparative genomic analysis of *Xanthomonas axonopodis* pv. *citri* str. A^w 12879 and *Xanthomonas axonopodis* pv. *citri* str. 5208 (Miami)

N. JALAN (1), N. Wang (1)
(1) Microbiology and Cell Science Dept, CREC, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S55

Xanthomonas axonopodis pv. *citri* (XAC) is the causal agent of citrus canker, which has a significant impact on citrus production. There are distinct types of canker that can be caused by various pathovars and variants of XAC. Because symptoms are generally similar, separation of these forms is based on host range and other phenotypic or genotypic characteristics of the strains. The Asiatic type of canker (Canker A), caused by Asian strain XAC, is by far the most widespread and severe form of the disease. Genetic variations of A-strain in Florida, resulted in local variants with either similar host range- XAC str. 5208 (Miami) or limited host range- XAC str. A^w 12879. Complete genome of XAC strain A^w 12879 was sequenced using 454-Titanium FLX sequencing (25x coverage) and Illumina paired-end 75bp Solexa run (288x coverage). The contigs were annotated and compared with previously published *Xanthomonas axonopodis* pv. *citri* str. 306 genome. Several singleton and shared features of the genome were identified, which hold potential for exploitation to get insights into virulence and host specificity of the strains. Solexa run was used to obtain the sequence of XAC strain 5208

Miami (367x coverage). Whole genome comparison with XAC str. 306 revealed that the strains share 99.99% sequence identity and only differ in 211 polymorphic sites. The polymorphisms offer a view into the adaptive evolution of the genus in different environments.

Characteristics of *Monilinia fructicola* isolates from decayed stone fruits in eastern West Virginia

W. J. JANISIEWICZ (1), A. R. Biggs (2), W. M. Jurick II (3), I. Vico (3), W. S. Conway (3)
(1) USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, U.S.A.; (2) West Virginia University, KTFRC, Kearneysville, WV, U.S.A.; (3) Food Quality Laboratory, USDA-ARS, BARC-East, Beltsville, MD, U.S.A.
Phytopathology 100:S56

Thirty eight isolates of *Monilinia fructicola* were obtained from decayed stone fruits (peach, plum, and nectarine) collected from trees growing in eleven eastern West Virginia orchards. The isolates were characterized phenotypically for growth characteristics, including: growth rate under different temperatures, sporulation, and resistance to fenbuconazole, the most commonly used preharvest fungicide in the region. There were five distinct culture phenotypes, ranging from albino to dark, melanized cultures. On PDA media, the growth rate per day of the isolates differed greatly at all temperature tested and ranged from 0.4 to 3.2 mm at 4°C, from 2.9 to 7.6 mm at 10°C, and from 6.5-15.5 at 24°C. Sporulation on peach agar at 24°C varied from profuse to no sporulation on three-day-old cultures, with some cultures sporulating only sparsely even on 10-day-old cultures. In spiral dilution tests, the ED_{50} for fenbuconazol ranged from 0.01 µg/µL to 0.137 µg/µL, indicating the development of resistance to the fungicide in some orchards. The identity of the isolates was confirmed genetically using sequences from the ITS region of the nuclear ribosomal RNA gene repeat. In addition, a sequence of a repetitive element, 'Mona', associated with resistance of *M. fructicola* to DMI fungicides, was detected in the isolates.

Development of infectious full-length cDNA clone of *Grapevine leafroll-associated virus* 3

S. JARUGULA (1), S. Gowda (2), W. O. Dawson (2), R. A. Naidu (1)
(1) Department of Plant Pathology, Washington State University, IAREC, Prosser, WA, U.S.A.; (2) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S56

Grapevine leafroll-associated virus 3 (GLRaV-3; genus *Ampelovirus* and family *Closteroviridae*) is associated with grapevine leafroll disease (GLRD). Different segments of the 18,498 nucleotide genomic RNA of GLRaV-3 were amplified using double-stranded RNA isolated from virus-infected grapevines showing GLRD symptoms. A multistep cloning strategy was used to assemble full-length genomic cDNA and its integrity was verified by sequencing. The full-length cDNA was cloned into an agro-binary vector, pCambia1380, between an enhanced 35S promoter and NOS poly-A terminator. Ribozyme sequence was added at the 3' termini of the cDNA clone to enhance infectivity. Infectivity of several cDNA constructs in *Nicotiana benthamiana* leaves was tested by agro-infiltration assays in the presence of RNA silencing suppressors. Total RNA analyzed by Northern blot hybridization using gene-specific non-radioactive riboprobes showed that six of the ten putative 3'-coterminal subgenomic (sg) RNAs were abundantly present only in leaves co-infiltrated with GLRaV-3 and silencing suppressor constructs. The sgRNA profile was similar to that obtained from total RNA extracted from grapevines naturally infected with GLRaV-3. Filamentous virion particles ranging from 1000–2000 nm long were observed in agro-infiltrated leaves, further demonstrating infectivity of agro-infiltrated cDNA clone. Availability of infectious cDNA clone enables to study molecular biology of GLRaV-3 and etiology of GLRD.

Xanthomonas* type III secretion system based analysis of candidate effectors from *Blumeria graminis* f. sp. *hordei

A. S. JAYASENA (1), Y. Meng (1), L. Bindschedler (2), P. Spanu (3), R. Wise (4), A. Bogdanove (1)
(1) Iowa State University, Ames, IA, U.S.A.; (2) University of Reading, Reading, UNITED KINGDOM; (3) Imperial College London, London, UNITED KINGDOM; (4) USDA-ARS / Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S56

Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* (*Bgh*) is a major disease of barley. Better understanding the *Bgh*-barley interaction will facilitate new control strategies. However, the obligate biotrophic lifestyle of *Bgh* hinders genetic studies. We have used the *Xanthomonas* type III secretion (T3S) delivery system to examine the effects of *Bgh* effector candidates (BECs) in barley, maize and rice. Vector pYM5 was constructed to fuse BECs to the T3S signal *avrBS2*. Confirming the effectiveness of this approach, CI16149 (*Mla10*) but not Manchuria (*mla10*) plants showed hypersensitive reaction (HR) to *Xanthomonas campestris* pv. *armoraciae 756C* carrying pYM5: AVR_{a10}. Therefore a set of 7 haustorial proteins from *Bgh* DH14 was screened on a panel of near-isogenic barley lines carrying different *R* genes, and on maize, B73 and MO17, parents of the maize IBM mapping population. In preliminary experiments, we identified one putative HR elicitor in HOR11358 (*Mla9*), two BECs that elicited an HR-like phenotype in MO17 and one that produced an HR-like necrosis in B73. Our data suggest that the bacterial T3S based assay is an effective screening method for identifying and characterizing fungal effectors that elicit necrosis. Screening of BECs in a compatible interaction between *Xanthomonas oryzae* and rice is in progress to identify effectors that affect plant disease susceptibility.

Elimination of *Black raspberry necrosis virus* (BRNV) from *Rubus occidentalis* by *in vitro* thermotherapy

A. JEON (1), E. Cheong (1), R. Mock (1)
(1) USDA, ARS, NGRL, Beltsville, MD, U.S.A.
Phytopathology 100:S56

Plants of the genus *Rubus* entering the U.S. are considered 'prohibited' plant germplasm and must be tested for exotic pathogens in the USDA quarantine program. Pathogens detected must be eradicated from host plants prior to germplasm distribution. *Black raspberry necrosis virus* (BRNV) infection in black raspberry (*Rubus occidentalis* L) results in yield and quality losses. Axillary buds of black raspberry cv. Munger infected with BRNV were grown on an *in vitro* culture medium at 23°C (room temperature - RT) or in a heat treatment (HT) regime of 4-hr periods of alternating 29°C/38°C with a 14 hr photoperiod. *In vitro* explants, removed from heat after 5 weeks and bi-weekly thereafter up to 13 weeks, were tested for BRNV using RT-PCR. All explants with heat treatments of 5–13 weeks tested free of BRNV. The explants were transplanted to soil, and leaf tissue was tested at monthly intervals. After two months in soil culture all explants have tested negative for BRNV. Testing of these and additional BRNV-HT plants will continue, to verify pathogen elimination. Introduction of the anti-viral chemical ribavirin (15mg/L) to the medium proved fatal to all explants (RT and HT). Rubus sp. plants with other viruses are being tested to determine optimum pathogen elimination protocols. This protocol will aid in the processing of valuable genetic resources through quarantine, and in broadening the genepool from which breeders can develop new and improved varieties.

Role of photoreceptors in R protein-mediated resistance to *Turnip crinkle virus*

R. JEONG (1), A. Chandra-Shekara (2), A. Kachroo (1), P. Kachroo (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) Dow AgroSciences, Indianapolis, IN, U.S.A.
Phytopathology 100:S56

Light harvested by plants is essential for the survival of most life forms. This light-perception ability requires the activities of proteins termed photoreceptors. In addition to various growth and developmental processes, light also plays a role in plant defense against pathogens and is required for activation of several defense genes and regulation of the cell death response. However, the molecular or biochemical basis of light modulated regulation of defense signaling is largely unclear. Previously we have shown that incompatible interaction between Arabidopsis-Turnip Crinkle Virus (TCV) pathosystem is dependent on light. Resistance to TCV is also dependent on the Resistance (R) protein, HRT. To determine the molecular and biochemical basis of light-dependent defense pathway, we studied the role of various photoreceptors in HRT-mediated resistance to TCV, HRT protein levels and its localization. Interestingly, mutation in certain photoreceptors led to degradation of HRT via a proteosome-dependent pathway and resulted in susceptibility to TCV. Exogenous application of salicylic acid induced transcription of HRT, which restored HRT levels in some, but not all, mutant backgrounds. These results show that different photoreceptors function distinctly in maintaining post-transcriptional stability of HRT. The current focus is to determine biochemical basis of photoreceptor-mediated stability of R proteins.

Suppression of sheath blight of rice by cow urine

R. JEYARAMAN (1)
(1) Annamalai University, Annamalainagar, Tamil Nadu, INDIA
Phytopathology 100:S56

Most rice growing countries are infested with *Rhizoctonia solani*, the causal agent of sheath blight. Although fungicides control the disease, they are not always economically or environmentally sustainable. Present activities to find alternatives that are safe to humans and environment. Utilization of cattle urine may provide an alternative for disease management. Studies were conducted to determine the stages of cow urine to control sheath blight of rice. Cow urine, bull urine, and bullock urine completely inhibit the growth of *R. solani* under in vitro. The toxicity of urine was not affected during storage.

Urine sprayed at 200 liter ha^{-1} was found significantly reduced the disease severity and increases the grain yield under greenhouse and field conditions. Urine treated plants showed more number of fungal and bacterial populations. Species of *Bacillus*, *Pseudomonas*, and *Streptomyces* were identified in urine treated plants. Urine treated plants showed enhanced synthesis of phenol and activities of phenylalanine ammonia lyase, peroxidase, β-1,3-glucanase, and chitinase relative to control. Our in vitro tests demonstrated that cow urine contains ammonia and nitrous acid are highly toxic to *R. solani* and, therefore, at least partially responsible for pathogen inhibition in urine treated rice plants. Thus, cow urine has the potential to suppress *R. solani* through chemical and microbial agents.

A new fungicide for control of *Phytophthora capsici* on vegetable crops
P. JI (1), J. Yin (1), M. Purvis (1), A. S. Csinos (1), L. J. Newsom (2)
(1) Department of Plant Pathology, University of Georgia, Tifton, GA, U.S.A.; (2) BASF Corporation, Tifton, GA, U.S.A.
Phytopathology 100:S57

Phytophthora blight, incited by *Phytophthora capsici*, causes severe yield and quality losses in production of peppers, cucurbits and other vegetable crops. Application of effective chemical fungicides continues to be a significant component in integrated management of this disease. A new fungicide, Zampro®, was evaluated in laboratory, greenhouse and field studies for control of *P. capsici*. In lab studies, EC_{50} values of this product in suppressing mycelial growth, zoospore germination and sporangium production averaged 0.69, 0.13 and 0.23 ppm, respectively. Foliar applications of Zampro at 5 to 25 ppm under greenhouse conditions significantly reduced Phytophthora blight severity on squash. Field studies were conducted in Tifton, GA, to evaluate Zampro for control of Phytophthora blight on squash and bell pepper. In 2008 studies, soil treatment with mefenoxam in conjunction with foliar sprays of Zampro proved most effective and provided greater disease suppression compared with mefenoxam applied alone. In 2009 field trials, Zampro and fluopicolide provided the greatest disease reductions among the treatments tested. Squash and pepper yield was significantly increased by Zampro treatments, compared with the non-treated control, in both years. The results suggest that Zampro has the promise to be used as an effective component in integrated programs for managing Phytophthora blight on vegetables. Zampro is expected to be registered by the US EPA in 2012.

Morphological and physiological alteration of maize root architectures on drought stress
T. JIANG (1), B. Scully (1), R. Kemerait (2), R. D. Lee (3), B. Guo (1)
(1) USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA, U.S.A.; (2) Department of Plant Pathology, the University of Georgia, Tifton, GA, U.S.A.; (3) Department of Crop and Soil Sciences, the University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S57

Drought tolerance is a complex agronomic trait and root characteristics logically play an important role in determining the response of plants to drought stress. Studies were conducted to investigate genotypic variations in morphological and physiological responses of roots to drought stress in corn. Two inbred lines, Lo964 and Lo1016, were planted in the field, greenhouse, and in the laboratory growth chamber for examination of the morphological and physiological alteration of root traits under drought stress versus no stress (well-water) conditions. The results revealed that Lo964 had a strong lateral root system, a high root/shoot ratio, and a high production of ABA in comparison with Lo1016 under drought stressed condition. The root systems of Lo1016 were much shallower and smaller than those of Lo964 under water and drought conditions. After 7 d of drought treatment, fresh root weights were significantly lower than that of well-watered plants for both Lo964 and Lo1016. ABA synthesis increased in both Lo964 and Lo1016 under drought stresses. The ABA contents increased by 9.6 and 3.1 times in the leaves of Lo964 and Lo1016, and 1.8 and 1.2 times in the root of Lo964 and Lo1016, respectively. Myo-inositol 1-phosphate synthase (MIPS) gene expression was 7 times higher in leaves of Lo964 than that of Lo1016 under well-water condition, which was decreased significantly to the level in Lo1016 under drought-stressed conditions.

Effects of surfactants on conidial germination of *Myrothecium verrucaria*
X. JIN (1)
(1) USDA ARS, Stoneville, MS, U.S.A.
Phytopathology 100:S57

Myrothecium verrucaria has been employed as a unique biological control agent because it is highly effective against several annual and perennial weeds, including red vine, trumpet creeper, redroot pigweed, kudzu, hempsesbania and sicklepod. Although aerial conidia of *M. verrucaria* are hydrophilic, surfactants are still needed to improve the biological control efficacy on weeds, such as kudzu. The mechanism of surfactants, mainly wetting agents, such as Silwet L-77 that enhance bioherbicidal efficacy remains unclear. This study tested the effects of several commonly used surfactants on the germination of aerial conidia of *M. verrucaria* on PDA plates. Surfactants used in this study were Kinetic HV, Sorbitan Monolaurate, Tween 40, Silwet L-77 and Latron AG 98. Conidia were also suspended in DI water and then spread on PDA plates as control. Incubation was conducted at 25°C. Germination percents were taken at 6 hours and 9 hours, respectively. Results indicated that Silwet L-77 and Tween 40 significantly ($P < 0.05$) promoted initial germination in the first 6 hours when compared to the control. At 9 hours of germination process, there were no significant differences among all treatments. Quicker germination of conidia after application may contribute to the enhanced bioherbicidal efficacy.

First characterization of a new *Exserohilum* foliar disease on warm season turfgrasses
Y. JO (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S57

An *Exserohilum* sp. has been isolated from foliar diseases observed on bermudagrass (*Cynodon* sp.) and zoysiagrass (*Zoysia* sp.) fairways and putting greens at golf courses in Houston, Texas since 2007. Disease symptoms on individual leaves exhibited prominent elliptical black lesions along the margin. Symptoms on the closely mowed turfgrass appeared as dark brownish to black spots of about 5 cm in diameter. As the disease progressed, individual spots coalesced into larger, irregular patches. Koch's postulates were verified for pathogenicity of the fungus on warm season turfgrasses through experiments in the growth chamber, and confirmation of the etiological agent causing the foliar disease was achieved. Mycelium was dark gray to black on potato dextrose agar medium, and produced no sexual fruiting structure or conidia unlike other *Exserohilum* spp. The fungus grew best on corn meal agar medium, and showed an optimal growth at 25°C and pH 7. Phylogeny trees were constructed based on internal transcribed spacer (ITS), Brn1 (reductase gene involved in melanin biosynthesis) and glyceraldehyde-3-phosphate dehydrogenase (gpd) gene sequences using PHYLIP. The sequences of the fungus isolated in the study showed no complete matching but high similarity with other known *Exserohilum* spp., indicating the fungus associated with a novel foliar disease on warm season turfgrasses is a new species of *Exserohilum*.

Effect of diverse cropping systems on arbuscular mycorrhizal fungal community diversity and structure
E. G. JOHNSON (1), J. H. Graham (1), D. O. Chellemi (2)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) USDA-ARS-USHRL, Ft. Pierce, FL, U.S.A.
Phytopathology 100:S57

In agricultural systems, AMF are greatly affected by soil disruption, lack of host tissue, inhibition due to fertilization, and fumigation. To determine the effect of diverse crop production practices on AMF community structure and diversity, five tomato crop production systems consisting of bahiagrass pasture, conventional, continuous vegetation removal (disk fallow), organic, and undisturbed (weed fallow) were initiated. The plots were adjusted to the new management regime for three or four years. Tomato production occurred for the next two years and soil DNA samples were taken prior to cultivation and after harvest. AMF phylotypes based on sequence combined with nonparametric multivariate statistical analysis were used to compare community structure and diversity. Bahiagrass, weed fallow, and organic management support diverse, but different AMF communities with multiple phylotypes lacking described sporotypes, while disk fallow and conventional management systems greatly reduce AMF populations. Tomato cropping tends to shift AMF communities to a low diversity community with phylotypes containing species that sporulate prolifically. After one year organic and conventional AMF communities are indistinguishable. Weed fallow communities are more resilient in absence of stress while bahiagrass communities are very stable. Comparison of the AMF communities in these cropping systems to environmental, disease, and yield data is underway.

Role of *Soybean mosaic virus* (SMV) genes in seed transmission in soybean
S. JOSSEY (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S57

SMV infection can cause severe crop losses and seed coat mottling. In North America, seed transmission serves as the primary source of inoculum for SMV as there are very few alternative hosts for the pathogen. Secondary spread of the virus is by aphids. To study the role of SMV coding regions in seed transmission, recombinant viruses were constructed using infectious clones of strains SMV 413 and SMV G2, which show high and low seed transmission, respectively. Using two restriction enzyme sites common in the two strains, reciprocal recombinants were made between the 5' and 3' regions

of the genomes of SMV 413 and SMV G2. Site-specific mutagenesis was used to create a recombinant virus in which the helper component proteinase of SMV 413 was replaced with that of SMV G2. The amino acid sequence of SMV G2 coat protein (CP) lacks a motif, DAG, which is important for aphid transmission. The role of this motif in seed transmission was examined by mutating the DAG motif in SMV 413 to DAD. Soybean seedlings, cv. 'Itachi', were inoculated with recombinant viruses, and seed transmission was assayed using seed grow-out tests. Seed transmission rates of 20 to 40% were recorded for SMV 413 and 0% for SMV G2. Recombinant viruses in which the 5' and 3' regions had been exchanged between the two strains failed to show any seed transmission. The single amino acid change in the DAG motif of the CP of SMV 413 reduced seed transmission by about 57%.

Purification and biochemical characterization of a polygalacturonase produced during Penicillium solitum decay of 'Anjou' pear fruit

W. JURICK (1), I. Vico (1), B. Whitaker (1), V. Gaskins (1), W. J. Janisiewicz (2), W. Conway (1)
(1) USDA ARS - Food Quality Laboratory, Beltsville, MD, U.S.A.; (2) USDA-ARS, AFRS, Kearneysville, WV, U.S.A.
Phytopathology 100:S58

Polygalacturonase (PG) was isolated and purified from Penicillium solitum-decayed 'Anjou' pear fruit. Ammonium sulfate precipitation, followed by gel filtration and cation exchange chromatography, were used to purify the enzyme. Both chromatographic methods revealed a single peak corresponding to PG activity. The enzyme had a molecular mass of 43 kDa and a pI of 5.3. PG activity was not associated with a glycosylated protein and the enzyme was active from pH 3.5 to 6, with an optimum at 4.5. The greatest PG activity was observed at 50°C but was also detectable at 0, 5, 10, 20, 37, and 70°C. In vitro PG activity was inhibited by addition of the divalent cations Ca, Mg, Mn, and Fe to varying levels. Thin-layer chromatographic analysis of hydrolysis products showed that the enzyme exhibits endo and exo activity, and is unable to cleave dimer or trimers of polygalacturonate. The purified PG macerated fruit in vitro and produced ~1.2 fold more soluble polyuronides on pear than on apple tissue. This is the first report of the production of a PG by P. solitum during postharvest decay of pear fruit, which is biochemically distinct from the previously characterized PG produced during apple fruit decay by the same fungus.

***Sclerotinia sclerotiorum* induces redox changes in the host cellular environment via oxalic acid**

M. KABBAGE (1), B. Williams (1), H. Kim (1), R. Britt (1), M. Dickman (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S58

The necrotrophic fungal pathogen *Sclerotinia sclerotiorum* produces the non-specific phytotoxin and pathogenicity factor, oxalic acid (OA). Transgenic plants expressing a redox-regulated GFP reporter, provided real-time evidence that Sclerotinia initially induces reducing conditions that suppresses the host oxidative burst and callose deposition, but subsequently promotes plant ROS generation leading to programmed cell death. Our non-pathogenic OA^- mutant strain is unable to alter host redox status, however chemical induction of reducing conditions in host cells with DTT, remarkably restores its ability to cause disease. OA thus appears to have dual opposing functions, by creating reducing conditions, OA inhibits the plant oxidative burst defense response and cell death much like in biotrophic interactions, and then subsequently promotes cell death and disease. The reduction of the host cellular environment may be a key strategy for establishment of necrotrophic fungal infection.

AtBAG7, an *Arabidopsis* Bcl2-associated athanogene resides in the endoplasmic reticulum and is involved in the unfolded protein response

M. KABBAGE (1), B. Williams (1), R. Britt (1), M. Dickman (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S58

The Bcl-2-associated athanogene (BAG) family is an evolutionarily conserved, multifunctional group of co-chaperones that perform diverse cellular functions ranging from proliferation to growth arrest and cell death in yeast, mammals and recently plants. The Arabidopsis genome contains seven homologs of the BAG family, including four with domain organization similar to animal BAGs. In the present study we show that AtBAG7 is a uniquely localized Endoplasmic Reticulum BAG that is necessary for the proper maintenance of the Unfolded Protein Response (UPR). AtBAG7 was shown to directly interact in vivo with the molecular chaperone, AtBiP2, by Bimolecular Fluorescence Complementation (BiFC) assays, and confirmed by yeast two hybrid assay. Treatment with an inducer of UPR, Tunicamycin (Tm), resulted in accelerated cell death of AtBAG7 null mutants. Furthermore, AtBAG7 knockouts were sensitive to known ER-stress stimuli, heat and cold. Heat sensitivity was successfully reverted in these knockouts to the wild-type phenotype with the addition of the chemical chaperone, Tauroursodexycholic acid (TUDCA). Real-time PCR of ER-stress proteins indicated that the expression of the heat shock protein, AtBiP3, is selectively up-regulated in AtBAG7 null mutants upon heat and cold stress. Our results reveal an unexpected diversity of the plants BAG gene family and suggest that AtBAG7 is essential component of the UPR during heat and cold tolerance, thus confirming the cytoprotective role of plant BAGs.

Effects of silicon amendment on diseases caused by *Mycosphaerella fijiensis* and *Cylindrocladium spathiphylli* in banana

L. KABLAN (1), M. Vermeire (1), J. Risede (2), M. Dorel (2), B. Delvaux (1), A. Legreve (1)
(1) Université catholique de Louvain, Louvain-la-Neuve, BELGIUM; (2) CIRAD-FLHOR, Capesterre-Belle-Eau, GUADELOUPE
Phytopathology 100:S58

Silicon (Si) is known to have many positive effects in defending plants against important pathogens. The effects of Si amendment in banana, an Si-accumulating plant, on its susceptibility to the airborne fungus *Mycosphaerella fijiensis* and to the soilborne fungus *Cylindrocladium spathiphylli* were studied under controlled conditions. The four last fully unfolded leaves of adult banana (*Musa acuminata*, cv Grande Naine) plants grown under a hydroponic culture system in the presence of Si (1,66mM) or not, were inoculated with *M. fijiensis* by spraying conidial suspensions or by brushing mycelial fragments. The foliar symptoms increased more rapidly on the non Si-supplied plants than on the Si-supplied plants and the severity was weaker on plants grown in the presence of Si. In another experiment conducted in the greenhouse, ten week-old plantlets supplied or not supplied with 2 mM of Si were inoculated by dipping the root system into a conidial suspension of *C. spathiphylli* and then transplanted in a Si-deficient ferralsol. Two weeks after inoculation, the root lesion severity of each plant was assessed using the image analysis program WinRHIZO. A reduction of about 50% of the root necrosis was observed for the Si-supplied plants compared with the non Si-supplied plants. The Si amendment also alleviated the growth reduction caused by the pathogen. These results suggest that Si might have a substantial role as a sustainable and non-pollutant means of managing important fungal diseases in banana.

Oleate-regulated signaling and plant defense

P. Kachroo (1), A. Kachroo (1), M. K. MANDAL (1)
(1) University of Kentucky, Dept. of Plant Pathology, Lexington, KY, U.S.A.
Phytopathology 100:S58

Oleate-regulated signaling and plant defenseMihir K Mandal, Aardra Kachroo, Pradeep Kachroo Department of Plant Pathology, University of Kentucky, Lexington, KY 40546Oleic acid (18:1) is one of the major monounsaturated fatty acid (FA) which plays an important regulatory role in animal cells. In plants, changes in the levels of 18:1 results in the alteration of salicylic acid (SA)- and jasmonic acid (JA)-mediated defense responses. This is evident in the Arabidopsis ssi2/fab2 mutant, which encodes a defective stearoyl-acyl carrier protein-desaturase (S-ACP-DES) and consequently accumulates high levels of stearic acid (18:0) and low levels of 18:1. Consequently replenishing 18:1 levels results in restoration of wild-type-like signaling in the ssi2 mutant. We have identified several genes, which either participate in the prokaryotic fatty acid (FA) or generalized defense pathways and loss-of-function of which restores various phenotypes in ssi2 plants. A reduction in 18:1 levels induces defense response by upregulating the expression of several R genes in an SA-independent manner (1). More recently, we have shown that 18:1 regulated induction of R proteins is dependent on EDS1 or SA and that EDS1 and SA act in a redundant manner to regulate R protein derived signaling regardless of the structure of R protein. Further characterization has led to the isolation of several 18:1 binding proteins, whose enzymatic activities are regulated by 18:1 levels. Detailed analysis of these proteins will be presented.

Functional analysis of soybean genes for a role in soybean cyst nematode resistance

P. KAITHERI KANDOTH (1), N. Ithal (1), J. Recknor (2), D. Nettleton (2), T. Maier (3), T. Baum (3), M. G. Mitchum (1)
(1) Division of Plant Sciences, Bond Life Science Center, University of Missouri, Columbia, MO, U.S.A.; (2) Department of Statistics, Iowa State University, Ames, IA, U.S.A.; (3) Department of Plant Patholgy, Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S58

Soybean cyst nematode (SCN ; *Heterodera glycines*) is one of the most important pathogens of soybean. The resistance against this pathogen is controlled by two major QTLs, Rhg1 and Rhg4, in soybean. In order to better understand the resistance mechanism against this pathogen, we performed comparative transcript profiling of laser-microdissected SCN feeding sites (syncytia) isolated from resistant and susceptible soybean NILs differing at the Rhg1 locus. This resulted in a highly specific library of differentially expressed genes in syncytia undergoing a resistant reaction and identified a

number of genes potentially involved in the resistance phenotype against this pathogen. Also, with the recent release of the complete soybean genome sequence, it is now possible to identify soybean homologs of known plant defense gene and test them for a role in the resistant reaction against this pathogen. A third pool of genes includes those mapped to resistance QTL loci. We are undertaking a large scale functional analysis of these genes in hairy roots by RNAi in composite plants of soybean. Moreover, the differentially expressed gene set is a suitable library for identifying nematode-inducible promoters which will be useful for highly targeted syncytia-specific gene silencing and overexpression studies.

Reaction to *Plasmodiophora brassicae*, pathotype 6 in lines of *Brassica* species

K. Kalpana (1), M. MCDONALD (1), B. D. Gossen (2)
(1) University of Guelph, Guelph, ON, CANADA; (2) Agriculture and Agri-Food Canada, Saskatoon, SK, CANADA
Phytopathology 100:S59

Clubroot of Brassica spp., caused by *Plasmodiophora brassicae* Woronin, is an important disease of vegetable Brassica crops worldwide, and has spread rapidly on canola (*B. napus*) in western Canada since first reported in 2003. Trials to evaluate clubroot incidence and severity on lines of selected *Brassica* spp. were established on naturally infested organic soil (pH 6.7) in 2008 and 2009 near Bradford, Ontario, Canada. Pathoptype 6 is predominant at this site. The study focused on the Rapid Cycling Brassica Collection (RCBC), including lines of *B. carinata*, *B. juncea*, *B. napus*, *B. nigra*, *B. oleracea*, *B. rapa*, and *Raphanus sativum*), which have potential as model systems for controlled environment studies and as differential hosts in pathotype assessment, but also included lines of canola and Asian vegetables (*B. rapa*) such as pak choy (var. *communis*), Chinese flowering cabbage (var. *utilis*) and napa cabbage (ssp. *Pekinensis*). Clubroot incidence and severity were higher in 2008 than in 2009, but the pattern of response was similar each year. Several of the RCBC lines were susceptible to pathotype 6, as were all of the Asian vegetables. RCBC lines of *B. carinata* and *B. juncea*, as well as pak choy and flowering cabbage, are good candidates for use as model crops. The susceptible canola lines will be useful for studies with pathotype 6, such as studies of fungicides and symptom development. Canola lines 5202 LL and 04- 2 and napa cabbage Deneko had no symptoms of clubroot.

Characterization of the antibacterial peptide herbicolin I biosynthetic operon in the fire blight biocontrol agent *Pantoea vagans* C9-1

T. Kamber (1), T. Lansdell (2), T. H. Smits (1), V. O. Stockwell (3), C. ISHIMARU (4), B. Duffy (1)
(1) Agroscope Changins-Wädenswil ACW, Wädenswil, SWITZERLAND; (2) Michigan State University, East Lansing, MN, U.S.A.; (3) Oregon State University, Corvallis, OR, U.S.A.; (4) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 100:S59

Pantoea vagans strain C9-1 (ex. *P. agglomerans*, *E. herbicola*) is one of the most effective and reliable biological control agents against fire blight, caused by *Erwinia amylovora*. It is registered for commercial use in the U.S.A. and Canada as BlightBan C9-1 (Nufarms Americas). Understanding C9-1 mechanisms of action is critical for improving biocontrol performance and regulatory evaluation in Europe. Competition and production of the antibiotic pantocin A are known mechanisms of pathogen suppression in the infection court (flower stigmas). We have characterized the peptide antibiotic herbicolin I (syn. dapdiamide E), which may also contribute to C9-1 biocontrol activity. A plasposon library was screened using novel pathogen biosensors. Insertion sequences for 16 herbicolin I-negative mutants were used to locate the herbicolin I operon in our complete genome of C9-1. The operon consists of 10 ORFs on a 166 kb plasmid. Comparative genomics identified a homologous gene cluster in *Serratia proteamaculans* 568, and absence in our sequence of the commercial strain *P. agglomerans* E325 (Northwest Agricultural Products). Prevalence of herbicolin I and diversity of biosynthetic genes among *Pantoea* biocontrol, environmental and clinical isolates, and pathogen resistance will be presented.

Microbial Rosetta Stone Central Agricultural Database: A database for high consequence plant pathogens

S. KAMENIDOU (1), A. Simonson (2), R. Jain (2), K. Hari (2), J. Robertson (3), J. Fletcher (1)
(1) Oklahoma State University, National Institute of Microbial Forensics & Food and Agricultural Biosecurity, Stillwater, OK, U.S.A.; (2) cBIO Inc., Freemont, CA, U.S.A.; (3) Federal Bureau of Investigation, Quantico, VA, U.S.A.
Phytopathology 100:S59

Plant pathogens, if used as agents of biowarfare, bioterrorism or biocrime, could have a serious impact on the U.S. food supply, environment and society. The Microbial Rosetta Stone (MRS) Central, a database developed for use by federal investigators, contains key information on high priority pathogens of humans and animals. The goal of this work was to select and provide curated information for 100 high consequence plant pathogens for the establishment of a MRS Central Agricultural Database. Plant pathogens were chosen based on their potential for damage to U.S agricultural and natural ecosystems, their inclusion on several existing plant pathogen threat lists and their recommendation by experts in the American Phytopathological Society's Microbial Forensics Interest Group (representing academia, government and industry). Information on these pathogens was collected using a multiple database search and keyword curation was performed for categories of taxonomy, synonyms, symptoms, detection and diagnosis, laboratory and field protocols, sample collection, geographic distribution, plant hosts, insect vectors and epidemiology. The resulting MRS Central Agricultural Database links curated data for high consequence plant pathogens and important plant diseases to relevant scientific literature and internet resources, thereby providing law enforcement, forensic and intelligence communities with an additional tool to counteract agricultural emergencies.

Development of *Cucumber mosaic virus* coat protein- and replicase-mediated resistant *Gladiolus* plants

K. Kamo (1), R. JORDAN (2), M. Guaragna (2), H. Hsu (1), P. Ueng (2)
(1) Floral and Nursery Plants Research Unit, USDA-ARS, U.S. National Arboretum, Beltsville, MD, U.S.A.; (2) Molecular Plant Pathology Lab, PSI, USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S59

Cucumber mosaic virus (CMV) is one of the most important plant viruses because it infects about 1000 plant species, including food crops and ornamentals. Infection of various flower bulb crops with CMV results in dramatic streaking of the flowers making the flowers unmarketable, and infected plants have decreased vigor resulting in a poor bulb yield. There are two subgroups of CMV (I and II) that are distinguished by serotype, biology, and molecular analysis. Transgenic *Gladiolus* plants that contain either CMV subgroup I coat protein (CMV CP I), subgroup II coat protein (CMV CP II), subgroup I replicase (CMV Rep), a combination of the CMV CP I and CMV CP II, or a combination of the CMV CP II and CMV Rep genes were developed. These plants were multiplied *in vitro* and challenged with purified CMV I and II *Gladiolus* isolates using a hand-held gene gun. Three out of 19 independently transformed plants expressing the CMV Rep gene under control of the duplicated CaMV 35S promoter were found to be resistant to CMV I. Three out of 21 independently transformed plants with the CMV CP II gene under control of the *Arabidopsis UBQ3* promoter were resistant to CMV II. Eighteen independently transformed plants with either the CMV CP I or a combination of CMV CP I and CP II genes were found to be susceptible to both CMV I and II. This work will facilitate the evaluation of virus resistance in transgenic *Gladiolus* plants to yield improved floral quality and productivity.

Response of hard red spring wheat germplasm to the bacterial leaf streak pathogen (*Xanthomonas campestris* pv. *translucens*)

Y. R. KANDEL (1), L. E. Osborne (1), K. D. Glover (1), C. Tande (1)
(1) South Dakota State University, Brookings, SD, U.S.A.
Phytopathology 100:S59

Bacterial leaf streak, caused by *Xanthomonas campestris* pv. *translucens* has emerged as an important disease of wheat in the Northern Great Plains. The potential for loss is not well known in the U.S. however the disease has been indicated to cause potential yield losses up to 20% in Mexico. As with many foliar bacterial diseases, in-season control is difficult and development of resistant genotypes is an urgent need. Limited information is available on sources of resistance to this disease. The objective of the study was to evaluate spring wheat germplasm against bacterial leaf streak. Field experiments were conducted near Aurora and Watertown, SD in 2009. Forty five HRSW genotypes with diverse genetic backgrounds were inoculated at tillering stage with a virulent isolate (XtSD-017). Disease severity was assessed at 7-day intervals from heading through dough stage. Differences in disease severity were observed, although no genotypes were immune. Of the 45 genotypes, most were susceptible however line SD4205 showed a high level of resistance whereas SD4148 and SD4176 were moderately resistant. Resistant genotypes had slower disease progression with shorter streak length. Resistant genotypes identified in this study can potentially be used in breeding programs to incorporate resistance genes into adopted hard red spring wheat genotypes. Additionally, these findings may have implications for the further study of bacterial leaf streak resistance genes.

A *Ustilago maydis* homolog of *Aspergillus veA* is required for hyphal proliferation in maize

B. KARAKKAT (1), S. Covert (2)
(1) University of Georgia, Athens, GA, U.S.A.; (2) University of Georgia, Warnell School of Forestry and Natural Resources, Athens, GA, U.S.A.
Phytopathology 100:S59

Members of the fungal-specific *velvetA* (*veA*) gene family affect spore production in saprobic Ascomycetes. However, the functions of similar proteins in Basidiomycetes have not been established. We predicted that veA gene homologs in the basidiomycete plant pathogen *Ustilago maydis* might regulate spore formation, spore viability, and disease progression. To pursue these hypotheses, three *U. maydis* genes *Um00893*, *Um04203* and *Um01146*, were identified by BLAST searches as *veA* family members. Using a gene replacement strategy, deletion mutants were made in all three genes. None of the mutants showed any phenotypic alteration during yeast-like, *in vitro* growth. However, the Um00893 mutants failed to induce gall or teliospore formation in maize. Chlorazol staining of leaves infected with *Um00893* mutants revealed that the mutant hyphae did not proliferate normally during the early stages of infection. The *Um01146* mutants were able to induce galls, but had a slight decrease in virulence while the *Um04203* mutants were not affected in disease progression. These data suggest that at least one *veA* family member is essential for *U. maydis* virulence.

Effect of *Trichoderma harzianum* on soil-borne pathogens affecting French beans in Kenya
G. M. KARIUKI (1), Z. M. Kinyua (2)
(1) Kenyatta University, Nairobi, KENYA; (2) Kenya Agricultural Research Institute, Nairobi, KENYA
Phytopathology 100:S60

The effect of *Trichoderma harzianum* T-22 on French beans has been studied in Kenya. A total of four trials were carried out during a period of one year in Naivasha, Kenya (under greenhouse conditions) and Mwea, Kenya (under field conditions) to evaluate the efficacy of *T. harzianum* in the management of soil-borne diseases of French beans caused by *Fusarium*, *Pythium* and *Rhizoctonia* spp. Preliminary work had shown that both sites had high incidence of these pathogens. A total of six treatments were applied in each of these sites and the trial was repeated once in each site. The treatments were: 30 g of *T. harzianum* per 1000 planting bags, 15g *T. harzianum* per 1000 planting bags, 5g of *T. harzianum* per 1kg of seed (seed treatment), 15 g of rootgard per 1000 planting bags, 23 ml of carbendazim per 2000 planting bags and the untreated control. The second application for each of the treatments except for the carbendazim was done again after 35 days. There were significant differences in terms of root/shoot growth and root colonization by *T. harzianum* in all the treated plots. The study confirms that *T. harzianum* enhances root growth of plants, and therefore renders the soil-borne pathogens ineffective. Root samples that were taken for plant pathogen DNA analysis further confirmed that *T. harzianum* is antagonistic to the soil-borne pathogens.

Host-pathogen interactions in the Mustard-White rust pathosystem: Protein expression profiling
P. KAUR (1), K. Sivasithamparam (1), M. Barbetti (1)
(1) The University of Western Australia, Perth, AUSTRALIA
Phytopathology 100:S60

Albugo candida (white rust) is a serious disease of cruciferous vegetable and oilseed crops. Leaf and inflorescence infection causes yield losses up to 60% or more in India, and losses of up to 20% in Australia. Presently, canola-quality *B. juncea* is being developed to extend Brassica oilseed production to the lower rainfall areas of the southern Australian grainbelt. Unfortunately, the varieties of *B. juncea* available in Australia appear highly susceptible to white rust. To understand the biochemistry of the host-pathogen interaction a proteomic study was undertaken to analyse changes in protein expression levels at different time points following inoculation, during the interaction of *A. candida* with resistant and susceptible cultivars, respectively. Some key regulators contributing to host resistance towards *A. candida* were determined that can be used to design genetic markers to screen for resistance available in *B. juncea* germplasm. Transcriptional expression changes shown by some enzymes reflect on how the pathogen manipulates the host plant to access its resources/nutrient reserves. These results provide a new insight into the mechanisms of resistance in this pathosystem.

A new product for *Peronospora* and *Pseudoperonospora* control in ornamentals
R. J. KEESE (1), K. E. Kalmowitz (2), C. Palmer (3)
(1) BASF Corp., Res Triangle Park, NC, U.S.A.; (2) BASF Corp., Raleigh, NC, U.S.A.; (3) IR-4 Project, Rutgers, The State University of NJ, Princeton, NJ, U.S.A.
Phytopathology 100:S60

Orvego™ fungicide, with the active ingredients ametoctradin and dimethomorph, will be a new experimental entry into the ornamentals market. Orvego is a combination of two modes of action – a mitochondrial respiration inhibitor (FRAC Group 45) and a FRAC Group 40 cell wall synthesis inhibitor. This combination has also been shown in field trials to be an effective resistance management tool. The range of rates tested for ornamentals, including phytotoxicity testing, were between 11 and 56 fl oz/100 gal. Efficacy has been demonstrated in research trials across the country with universities and through the IR-4 program, against a number of downy mildews and excellent control is achieved with these rates. Crop safety at a 4X safety factor has been established in many ornamentals and a plant list will be reviewed. Efficacy studies on Coleus, deadnettle, rose and others will be discussed. Registration is expected in 2012.

Controlling powdery mildew in the greenhouse on hybrid *Cucurbita* seedlings used as rootstocks for grafting watermelon
A. P. KEINATH (1), G. V. Baccari (1), V. B. DuBose (1)
(1) Clemson University, Charleston, SC, U.S.A.
Phytopathology 100:S60

'Strong Tosa' interspecific hybrid squash (*Cucurbita moschata* x *C. maxima*) is used as a rootstock for grafting seedless watermelon scions. 'Strong Tosa' seedlings may become infected by powdery mildew *Podosphaera xanthii* before grafting or during the healing phase after grafting. The objective was to test 5 biofungicides, 6 conventional synthetic, and 3 organic-approved fungicides to prevent powdery mildew on 'Strong Tosa.' Fungicides at labeled rates per 935 l/ha water were applied to seedlings. In the first two experiments, seedlings were sprayed three times at 5-day intervals and exposed to powdery mildew inoculum continuously after the first application. In the second two experiments, seedlings were exposed to inoculum for 7 days, sprayed once, and held in a humidity chamber for 7 days to simulate healing conditions. Powdery mildew incidence and severity were greater with than without humidity. In all experiments, conventional fungicides were more effective than organic-approved fungicides, which were more effective than biofungicides. Hydrogen dioxide, skim milk, and thiophanate-methyl were ineffective compared to water. Penthiopyrad (LEM-17), cyprodinil plus fludioxonil, tebuconazole, and myclobutanil were very effective and did not differ from each other. Parafinic oil (JMS Stylet Oil), sulfur, and potassium bicarbonate were as effective as conventional fungicides in three of four experiments (P = 0.01). Fungicides should be applied before exposure to powdery mildew.

Effect essential oils on inhibition of *Phytophthora capsici*
E. KELLEY (1), J. Hao (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S60

Phytophthora capsici is an economically important pathogen that infects many plants, including cucurbit and solanaceous species. Laboratory studies were conducted to determine the effect of essential oils (bay, cinnamon leaf, clove bud, rosemary, and red thyme) on *P. capsici* (1) mycelial growth, (2) zoospore production, motility and mortality, and (3) oospore production. (1) A mycelial plug was transferred to a Petri plate containing V8 agar medium, amended with an essential oil (V8-O) and colony diameter was recorded after 3 days. (2) *P. capsici* was grown on V8-O plates for 5 days in the dark, followed by 3 days in constant light. Released zoospores were counted using a hemacytometer. Zoospore suspensions were treated with essential oils and aliquots were pipetted onto V8 plates, with colonies counted 2 days later. Treated zoospores were also viewed under a compound microscope for movement at 3, 10, 30, and 60 minutes. (3) Two strains of *P. capsici* with opposite mating types were co-cultured on V8-O plates, and oospore production was observed after 1 week. All tests were done utilizing 5 oil concentrations, in the range of 0.005 to 0.8 ul/ml. Effective concentration for 50% growth inhibition of the pathogen was calculated. At a concentration of 0.15 ul/ml, red thyme was the only oil to fully inhibit all mycelia growth, but bay clove bud and cinnamon leaf inhibited growth at 0.3 ul/ml. All oils suppressed the movement of zoospores within three minutes of treatment at 0.15 ul/ml.

Structural studies of filamentous plant viruses by X-ray fiber diffraction and cryo-electron microscopy
A. KENDALL (1), J. Groom (1), W. Bian (1), M. McDonald (1), D. Williams (1), P. Stewart (1), S. Ghabrial (2), G. Stubbs (1)
(1) Vanderbilt University, Nashville, TN, U.S.A.; (2) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S60

The filamentous plant viruses are important agricultural pathogens that cause significant economic losses, particularly in developing countries. Although there have been extensive genetic and plant pathological studies of these viruses, little detailed structural information has been available until recently. Using a combination of X-ray fiber diffraction and cryo-electron microscopy, we have produced the first medium resolution models of several filamentous plant viruses including the potexvirus potato virus X and the potyvirus soybean mosaic virus. We have also extended our studies to other filamentous plant viruses including the hordeiviruses and the closteroviruses. Improving the resolution of these models will allow us to phase our fiber diffraction data

and enable the production of high resolution models. These models will give us insight into viral-host interactions and virus assembly and disassembly, and will facilitate the use of these viruses in biotechnological applications. Supported by NSF grant MCB-0743931.

Screening for differential resistance responses to *Phakopsora pachyrhizi* between *Rpp3*, *Rpp?*(Hyuuga), and 12 additional soybean accessions

M. D. KENDRICK (1), K. F. Pedley (1), R. D. Frederick (1), D. L. Hyten (2), P. B. Cregan (2), D. K. Harris (3), B. Ha (3), H. R. Boerma (3)
(1) USDA-ARS, Fort Detrick, MD, U.S.A.; (2) USDA-ARS, Beltsville, MD, U.S.A.; (3) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S61

Asian soybean rust (ASR) is an economically significant disease caused by the fungus *Phakopsora pachyrhizi*. Five soybean genes that confer resistance to specific isolates of *P. pachyrhizi* (*Rpp1 – Rpp5*) were previously identified. More recently, the soybean cultivar Hyuuga (PI506764) was found to be resistant to field isolates of the pathogen. The genes *Rpp?*(*Hyuuga*) and *Rpp3* map to the same region of chromosome 6, between markers Satt460 and Satt307, and Satt460 and Sat_263 respectively. Twelve additional soybean accessions with resistance to *P. pachyrhizi* and mapping within 5 cM of the *Rpp3*/*Rpp?*(*Hyuuga*) locus have been identified by a bulk-segregant approach. It is unknown whether the resistance genes in PI506764(Hyuuga), PI462312(*Rpp3*), and these 12 lines are identical, allelic, or independent genes. Previously it was reported that PI462312 (*Rpp3*) and PI506764 (Hyuuga) responded similarly when inoculated with 10 *P. pachyrhizi* isolates. However, when challenged with a Brazilian isolate, *Rpp3* plants were susceptible while *Rpp*?(Hyuuga) were resistant, leading to the possibility that 'Hyuuga' may carry a unique allele at the *Rpp3* locus or another resistance gene. To further characterize these 14 lines, we inoculated each with additional isolates. The differential responses that we observed suggest that resistance is conditioned either by two closely linked loci or two different alleles at the same locus.

USDA-APHIS plant pest permitting policy pertaining to containment facilities for plant pathogens

M. J. KENNEY (1)
(1) USDA APHIS, Riverdale, MD, U.S.A.
Phytopathology 100:S61

The research community has interest in new and emerging plant diseases, agrobioterrorism, and exotic biocontrol organisms; research in these areas frequently must be conducted in containment facilities in order to safeguard American agriculture and the environment. The Animal and Plant Health Inspection Service (APHIS) recently implemented several new policies and standard conditions pertaining to the application, inspection, approval and maintenance of containment facilities. The level of biocontainment security required is based on risk of escape and possible establishment of plant pests. Containment facilities can consist of laboratories, growth chambers, and greenhouses, singly or combined. Containment facilities can be expensive to design, build, and maintain. APHIS provides assistance to permittees during this process; it also evaluates all containment facilities before permits are issued for pathogen research. Periodic re-evaluations are also required. Only a small percentage of the more than 2,000 APHIS-approved containment facilities are adequate to do work with high risk pathogens such as *Puccinia graminis* race UG99, *Phytophthora ramorum*, and plum pox virus. In addition, to ensuring that appropriate structural safeguards are established and maintained, there may be additional permits conditions that restrict the movement and use of plant pathogens.

Identification of the molecular make-up of the *Potato virus Y* strain PVY^{Z}

C. KERLAN (1), O. Nikolaeva (1), X. Hu (1), T. Meacham (1), S. Gray (2), A. Karasev (1)
(1) University of Idaho, Moscow, ID, U.S.A.; (2) USDA-ARS, Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S61

Potato virus Y (PVY) strains were defined by interactions with different resistance genes in potato cultivars. Five distinct strain groups were distinguished that caused local and/or systemic hypersensitive response in the genetic background with a corresponding *N* gene, these were PVY^{O}, PVY^{N}, PVY^{C}, PVY^{Z} and PVY^{E}. Here, we report that a recently found recombinant isolate PVY-L26 induces a hypersensitive response in a potato cultivar carrying the *Nz* gene, and is not recognized by two other resistance genes, *Ny* and *Nc*. These genetic responses in potato, combined with the inability of PVY-L26 to induce vein necrosis in tobacco, clearly define it as an isolate from the PVY^{Z} strain group and provides the first information on genome structure and sequence of this PVY^{Z} strain. The genome of PVY-L26 isolate displays typical features of $PVY^{EU\text{-}NTN}$ isolates, i.e. European NTN type with three recombinant junctions. Two PVY^{NTN} isolates elicited similar HR reactions in cv Maris Bard carrying *Nz* gene, but, unlike L26, induced vein necrosis in tobacco. Yukon Gold, a North American potato cultivar was shown to produce HR against infection with PVY^{NTN} isolates, as well as with L26 and, thus, to carry the *Nz* resistance gene. Based on genetic responses in potato cultivars carrying *Nz*, *Ny* and *Nc* genes, and molecular make-up of their genomes we propose to group PVY^{Z} and PVY^{NTN} isolates into a single $PVY^{Z/NTN}$ strain group.

CorA, a magnesium/nickel/cobalt transporter is required for full virulence in the soft rot pathogen, *Pectobacterium carotovorum*

C. KERSEY (1), C. K. Dumenyo (1)
(1) Tennessee State University, Nashville, TN, U.S.A.
Phytopathology 100:S61

Pectobacterium carotovorum (*Erwinia carotovora* subsp. *carotovora*) is a soft rot plant pathogen that produces tissue maceration in host by secreting exoenzymes that degrade polysaccharide-based plant cell wall components. Although many genes are known to be involved in the regulation of virulence and exoenzyme production, the function of approximately 20% of *P. carotovorum*'*s* predicted proteome still remains unknown. We have isolated a mini-Tn5 *lacZ*1 transposon mutant of *P. carotovorum Ecc* 71 (CKD_A12) that is altered in the production of exoenzymes and virulence. Compared to the parent, the mutant produced less extracellular protease (Prt), cellulose (Cel), pectate lyase (Pel) and polygalacturonase (Peh) and macerated less plant tissues. The mutated gene in CKD_A12 was identified as *corA*, a magnesium/nickel/cobalt transporter. The mutant phenotype was confirmed in parental strain *P. carotovorum Ecc* 71 by marker exchange inactivation of *corA*. The marker exchanged mutant was tested for exoenzyme levels, and as in mutant CKD_A12, Pel, Prt, Peh, and Cel levels were all reduced in the *corA* mutants. Complementation of both the original and the marker exchange *corA* mutant strains with a functional $corA^{+}$ gene from *P. carotovorum Ecc* 71 restored exoenzymes levels to parental levels and pathogenicity in celery and carrot. These results indicate that, like in *Salmonella enterica*, CorA is involved in the virulence of *P. carotovorum*.

Gene expression profiling of two Citrus cultivars in response to huanglongbing (HLB) using the Agilent Citrus custom microarray Chip

A. A. KHALAF (1), F. G. Gmitter (2), R. H. Brlansky (2), J. Fan (2), G. A. Moore (1)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Citrus Research and Education Center, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S61

'*Candidatus* Liberibacter', a phloem-limited bacterium, is associated with citrus greening disease also known as huanglongbing (HLB). HLB infects all citrus types and causes rapid decline of trees. There is no good source of genetic resistance to HLB in the genus 'Citrus'. A microarray experiment was designed to compare gene expression response of two citrus cultivars to Liberibacter infection, sweet orange (*C. sinensis*) that is extremely susceptible and rough lemon (*C. jambhiri*), a more tolerant variety according to field observations. Plants from both cultivars were graft-inoculated with HLB. A similar set of plants of the same size and age from each cultivar was mock graft-inoculated. RNA was extracted from leaves collected at four time-points post inoculation (0 time, 9, 17, 27 wpi) from both inoculated sweet orange and mock inoculated plants. The arrays were repeated three times with different independent biological replicates from infected and mock-inoculated plants of both cultivars. The resultant complimentary RNA (cRNA) was hybridized to our custom Citrus Agilent GeneChip Array (4x44K format). Gene expression analysis results will be presented.

Synergistic agents to reduce fungicide resistance and health risks

N. I. KHAN (1), J. A. Trogolo (1)
(1) Agion-Technology Inc., Wakefield, MA, U.S.A.
Phytopathology 100:S61

Agion Technologies Inc. has developed highly effective microbiocides with levels of mixed metals up to 1,000 times lower than the early fungicides. Agion's formulation C200, may function as a stand alone biocide and may be most valuable as a powerful synergist with conventional fungicide thereby reducing the use levels and their environmental impact, as well as may result in efficacy against the resistant strains. Laboratory test results indicate that C200 alone at 100pm, 50 ppm or 25 ppm or combining with Mancozeb (EBDC) or Daconil (Cholorothalonil) at 50%, 25% or 12.5% can be as effective as Mancozeb or Daconil at 100% recommended dose level against both *Alternaria solani* and *Botrytis cineria* ($P > 0.05$). Combining C200 at 50 ppm and 25 ppm with Daconil or Mancozeb at 50% and 25% of there recommended doses showed additive or synergistic effect that was not significantly different from the 100% recommended dose of Mancozeb. In repeated detached tomato leaf bioassay experiments, C200 alone at 100ppm, or combined with Mancozeb 50% of the recommended dose showed significant reduction in mean disease severity over control ($P > 0.05$). There was no significant difference among, C200 at 100 ppm,

50 ppm, and 25 ppm, Mancozeb at 100%, 50% and 25% of the recommended dose, and the combination treatments where C200 100ppm was combined with Mancozeb at 50% or 25% of the recommended dose ($P > 0.05$). Greenhouse and field experiments will be performed in the next phase.

Prevalence of benzimidazole resistance in *Botrytis cinerea* isolates from Rose greenhouses in Center of Iran
P. KHAZAELI (1), H. Zamanizadeh (1), B. Morid (2), S. Hajmansoor (1)
(1) Department of Plant Pathology, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Department of Plant Protection, Takestan Branch, Islamic Azad University, Takestan, IRAN
Phytopathology 100:S62

Botrytis cinerea Pers. is a phytopathogenic fungus which causes grey mould on over 230 hosts. Development of fungicide resistance is now one of the major problems in plant disease control. The widespread use of fungicides, such as benzimidazoles, in the prevention or elimination of fungal attack has resulted in the appearance of resistant strains. This study was conducted to evaluate the sensitivity and resistance response to benzimidazole of 51 isolates of *B. cinerea* were obtained from Roses are cultivated under greenhouses in Tehran and Markazi provinces of Iran. The isolates were identified at the species level by their morphological characteristics and molecular method with used specific primer. Threshold concentrations for evaluating the resistance were 1 ppm for sensitive, 50 ppm for resistance and 500 ppm for high resistance. Results showed that 42% of isolates grew on PDA containing 500 ppm benomyl and showed the high resistance phenotypes, whereas all other isolates were inhibited by this concentration and grew on PDA containing 1 ppm benomyl and showed that sensitive phenotypes. According to the results, the resistance of *Botrytis cinerea* isolates to benzimidazole is increasing in rose producing greenhouses of Iran and this widespread occurrence of resistance to benzimidazole fungicides suggests that their efficacy against grey mould might be impaired and that more aggressive resistance management is needed.

A semi-automated system for quantitative analysis of *Meliodogyne* reproduction
J. KILCREASE (1), F. Solano (1), A. Rascon (1), S. Hanson (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 100:S62

Quantification of eggs produced by *Meloidogyne* sp. as an indicator of infection severity is a long accepted and practiced technique in nematology and plant pathology. Since resistance to *Meloidogyne* is often partial rather than complete, accurate quantitative measurements are essential for evaluation of resistance in plants. Current methods used to count eggs produced are tedious, time consuming, and prone to error. Herein we describe the assessment of several methods for quantifying *Meloidogyne* reproduction in plants and the development of a semi-automated system for quantification of nematode eggs. We also show the use of this system in the evaluation of infection severity in a nematode resistance development project. The system uses computer assisted image analysis to count eggs captured in micrographs of egg samples. Evaluation of several parameters demonstrates that this system is substantially faster and more accurate than manual counting methods. The system is also free of human bias that can skew results of manual counting schemes. The data presented here includes methods for extraction of eggs from infected plants and a description for how similar quantification systems can be set-up on a minimal budget.

Role of structural polysaccharides in the virulence and transmission of *Xylella fastidiosa*
N. KILLINY (1), R. Almeida (1)
(1) University of California-Berkeley, Berkeley, CA, U.S.A.
Phytopathology 100:S62

Xylella fastidiosa, is the causal agent of Peirce's disease in grape and other emergent diseases. It is transmitted by the xylem sap feeding sharpshooters where it colonizes the foregut. Previous work showed that, the host structural carbohydrates, pectin and glucan provoke significant changes in gene regulation resulting in inducing the transmission by vector. Here, we report the effect of the vector polysaccharide, chitin on *X. fastidiosa* growth. Similarly to pectin, chitin affects the gene regulation. It up-regulates the adhesion proteins and down-regulates the movement genes. Cells grown in the presence of chitin showed are more attached to glass rather than planktonic. Additionally, *X. fastidiosa* cells were able to form a biofilm on the citinaceous surface of insect wings and use chitin as a carbon source. We propose that chitin in insect foregut is an alternative environmental sensor to pectin. Our data show that, the environmental sensors (polysaccharides) and the diffusible signaling factor (DSF) contributes jointly in the gene regulation to produce attached and movable types of *X. fastidiosa* cells which are required to the virulence and transmission.

Molecular characterization of genes differentially expressed during conidiation by *Magnaporthe oryzae*
K. KIM (1), Y. Lee (1)
(1) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, SOUTH KOREA
Phytopathology 100:S62

Like most fungal pathogens, conidia (asexual spores) of *Magnaporthe oryzae* play a key role in disease cycle. Briefly, this includes conidial attachment to the surface of host plants, conidial germination, appressorial development, colonization of host cells, and asexual reproduction (conidiation) for conidium-mediated dissemination and new infections. This cycle occurs several times during rice-growing season, causing severe loss of rice production. Therefore, understanding the molecular mechanisms involved in conidiation would be a priority to develop novel strategies for disease management. However, relatively little is known about molecular mechanisms that regulate conidiation in *M. oryzae*. Recently, our lab revealed that a homeodomain transcription factor, MoHOX2 is a key regulator, specifically involved in conidiation. To better understand the molecular mechanism of conidiation-mediated by MoHOX2 in *M. oryzae*, we performed a microarray analysis using RNAs from conidiating and nonconidiating mycelia of the wild-type, and the conidiation-defective mutant, *ΔMohox2*. This analysis resulted in the identification of genes differentially expressed during conidation and in the conidiation-defective mutant. Many were revealed to encode potential regulators such as transcipriton factors, protein kinase, phosphatases, and etc. Ongoing works on identified genes from a microarray analysis will be presented.

Fsr1-interacting proteins in *Fusarium verticillioides* are required for stalk rot virulence on maize
J. KIM (1), C. Wang (1), B. D. Shaw (1), W. Shim (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S62

Maize stalk rot is a complicated disease primarily caused by fungal pathogens. Using *Fusarium verticillioides*, one of the key stalk rot pathogens, we discovered that a protein, designated Fsr1, plays an important role in stalk rot virulence. The predicted Fsr1 protein contains multiple protein-binding domains, and the coiled-coil (CC) domain in the N-terminus was determined essential for virulence. The CC domain is known to mediate protein-protein interactions, and our premise is that this interaction triggers downstream gene signaling associated with stalk rot virulence. The aim of this study was to identify putative Fsr1-binding proteins and determine their role in stalk rot virulence. In particular, we used the N-terminal region of Fsr1 as bait and performed yeast two-hybrid experiments. We identified two putative Fsr1-binding proteins, Wor1 and Pex14, in *F. verticillioides*, and these interactions were further verified through co-immunoprecipitation. For further characterization, we generated *wor1* and *pex14* knockout mutants, Δ*wor1* and Δ*pex14*, respectively. Interestingly, while both mutants showed reduced stalk rot virulence, they were not as severe as the symptom caused by *fsr1* deletion mutant. Our data suggest that Wor1 and Pex14 play a role in stalk rot virulence by interacting with Fsr1. Further in vivo confirmation of Fsr1-Wor1/Pex14 interactions with the use of fluorescent proteins are in progress.

Functional characterization of *FST1* from *Fusarium verticillioides*
H. KIM (1), C. P. Woloshuk (1)
(1) Purdue University, Department of Botany and Plant Pathology, West Lafayette, IN, U.S.A.
Phytopathology 100:S62

Fusarium verticillioides causes an ear rot disease in maize and produces the mycotoxin fumonisin B1 (FB1). *FST1*, a putative hexose transporter gene in *F. verticillioides*, is highly expressed in endosperm compared with germ. When inoculated onto ears, a disruption-mutant (Δ*fst1*) grew at half the rate as the wild type, disease symptoms were delayed, and no FB1 was detectable. A functional characterization of the *FST1* promoter revealed that regulation of *FST1* expression is similar to fumonisin (*FUM*) genes; the expression was the highest during growth on endosperm and repressed by high levels of ammonium. Fluorescence microscopy with a strain expressing a fluorescent-tagged FST1 suggested that FST1 is localized to the plasma membrane. Expression of FST1 failed to complement growth of a yeast strain that lacks hexose transporter genes. From the results, we hypothesize that FST1 functions as an environmental sensor that is important for initiating mycotoxin production and for colonization of maize kernels.

Stability and fitness of pyraclostrobin- and boscalid-resistant phenotypes in field isolates of *Botrytis cinerea* from apple

Y. KIM (1), C. Xiao (1)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.
Phytopathology 100:S63

Phenotype stability and fitness of pyraclostrobin (PYR)- and boscalid (BOS)-resistant isolates of *Botrytis cinerea* from apple were evaluated. Stability of resistance to PYR and BOS was determined after consecutive transfers on potato dextrose agar (PDA) or being cycled on apple fruit. Various fitness components including mycelial growth, osmotic sensitivity, conidial germination, pathogenicity and virulence on apple fruit, sporulation in vitro and in vivo and competitive ability on apple fruit were evaluated. After 20 and 10 transfers on PDA and 5 and 3 cycles on apple fruit at 20 and 0°C, respectively, resistance to PYR and BOS retained at the similar levels as the initial generation. There were no significant differences in mycelial growth, osmotic sensitivity, conidial germination and sporulation between resistant and sensitive isolates except that isolates resistant only to BOS produced fewer conidia in vitro. Resistant isolates were as pathogenic and virulent on apple fruit as sensitive isolates. After four disease cycles on apple fruit inoculated with a mixture of a sensitive isolate and one of the three resistant phenotypes, the frequency of PYR-resistant individuals was significantly decreased compared to the initial generation and no BOS-resistant individuals were detected. The results indicate that resistance to PYR and BOS in *B. cinerea* was stable and that PYR- and BOS-resistant isolates did not compete well with sensitive isolates in vivo.

Sensitivity to pyraclostrobin and boscalid in *Penicillium expansum* populations from apple in Washington State

Y. KIM (1), Y. Yin (1), C. Xiao (1)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.
Phytopathology 100:S63

Blue mold caused by *Penicillium expansum* is a major postharvest disease of apples. Pristine (pyraclostrobin + boscalid) as a preharvest treatment is effective in controlling blue mold. To establish baseline sensitivity to pyraclostrobin and boscalid, 50 isolates of *P. expansum* collected prior to the registration of Pristine were tested for sensitivity to the fungicides using conidial germination assays. EC_{50} values of pyraclostrobin ranged from 0.004 to 0.009 µg/ml, with a mean of 0.006 µg/ml. Boscalid only delayed conidial germination compared to the control while most conidia were able to germinate after 30 h of incubation at all concentrations (up to 100 µg/ml) tested. The minimum inhibitory concentration of pyraclostrobin was 1 µg/ml. To monitor resistance to pyraclostrobin, 145 isolates from decayed apples originating from orchards where Pristine had been used for 4 consecutive years were collected from a commercial packinghouse and screened for resistance to pyraclostrobin. EC_{50} values of pyraclostrobin for a subset of 30 isolates from the exposed population were determined. All exposed isolates remained sensitive to pyraclostrobin. There was no significant difference in mean EC_{50} values of pyraclostrobin between the baseline and exposed populations. The results indicated that boscalid was not effective against *P. expansum* and that there was no significant shift in sensitivity to pyraclostrobin in the exposed populations after 4-year use of the fungicide.

Cytological and genetic responses of near isogenic lines carrying rice blast resistance genes

H. KITO (1), K. S. Zenbayashi (1), T. Nakajima (1)
(1) National Agricultural Research Organization, Daisen, Akita, JAPAN
Phytopathology 100:S63

The partial resistance gene *Pi34* is proposed to confer durable resistance to blast in rice. To analyze the partial resistance cytologically, we observed penetration of *Magnaporthe oryzae* to rice leaf blades with whole cell clearing method (Koga and Kobayashi 1980). *Pi34*-NIL and Chubu32, which is a donor cultivar of *Pi34*, did not inhibit penetration of *M. oryzae*. We also investigated penetration and infection in rice by intact leaf sheath inoculation method (Koga *et al*. 2004). The epidermal cells of *Pi34*-NIL did not prevent *M. oryzae* from penetrating but infecting to adjacent rice cells. Cell death and accumulation of active peroxide in epidermal cells of *Pi34*-NIL under appressoria showed similarity to that of *Pib*-NIL. Consequently, *Pi34* presented common cytological phenotypes with *Pib*, which classified into the true resistance gene, except for penetration. To investigate mechanisms of the partial resistance genetically, transcriptome analysis was conducted with SuperSAGE method (Matsumura *et al*. 2003). Two tags, OMG-01 and OMG-02, presented distinct expression depending on the presence of *Pi34*. These cDNAs and genomic fragments corresponding to OMG-01 and OMG-02 were amplified and sequenced. OMG-01 and OMG-02 had two alleles in the cultivars carrying and not-carrying *Pi34*, respectively. All of them were expressed. Expression pattern of OMG-01 was constitutive, whereas that of OMG-02 was infection inducible. We speculated that OMG-02 was the primary candidate of Pi34.

Ametoctradin: A new Oomycete specific fungicide

K. KLAPPACH (1), K. Walker (2)
(1) BASF, Limburgerhof, GERMANY; (2) BASF, Res Triangle Park, NC, U.S.A.
Phytopathology 100:S63

Ametoctradin is a new Oomycete specific fungicide under development by BASF Corporation. Ametoctradin belongs to a new class of chemistry the triazolo-pyrimidylamines in FRAC group 45. It is a strong inhibitor of mitochondrial respiration in complex III and has shown to be very active against zoospores and zoosporangia. It is an excellent preventative material with a high affinity for the waxy layers of the leaf surface. In research trials, it has show excellent residual activity and rainfastness properties. Ametoctradin controls major plant pathogens from the Oomycete class of fungi, specifically downy mildews and Phytophthora spp. on vine, vegetable crops and ornamentals. Ametoctradin is very active against Plasmopara viticola in grape, Phytophthora infestans and a broad variety of downy mildews. The compound has a favorable toxicological and ecotoxicological profile. The active ingredient trade name for ametoctradin is Initium® Fungicide. EPA registration is expected in 2012.

Predicting field activity of experimental fungicides on Septoria leaf blotch of wheat using multiple regression modeling

C. J. KLITTICH (1), Y. Adelfinskaya (1), W. K. Brewster (1), C. Yao (1)
(1) Dow AgroSciences LLC, Indianapolis, IN, U.S.A.
Phytopathology 100:S63

Fungicide invention generally involves greenhouse testing to predict the efficacy of compounds in the field, but translation of activity from greenhouse to field can be skewed by many factors (rainfastness, UV stability, redistribution, etc.) in ways that are difficult to predict. We used multiple regression modeling to determine which biological and physiochemical measurements would best predict control of Septoria leaf blotch of wheat in the field. Nine compounds from a novel chemical class were tested side-by-side in field microplots in 2007. Field activity was well modeled (R2 adj. = 0.87) using only two factors; greenhouse protectant IC50 (Prob > |t| = 0.0005) and UV stability (T½ range 1-150 hr, Prob > |t| = 0.09). Other factors such as melting point (range 96-167 C) and log Kow (range 3.5-4.2) were not significant. To test the importance of UV stability to field activity, we selected compounds with a wider range of UV stability (T½ range 26-1300 hr) for field testing in 2008. Compounds with high UV stability were less active than the 2007 model predicted, and regression modeling of the 2008 data showed that, while greenhouse IC50 continued to be a strong predictor of field activity (Prob > |t| = 0.008), UV stability did not contribute significantly to the model (Prob > |t| = 0.25). We concluded that greenhouse activity best predicts the field control of Septoria leaf blotch by this chemistry, and that UV stability is not a consistent predictor.

Evidence that fern distortion syndrome is caused by fluorescent pseudomonads

J. W. KLOEPPER (1), F. Saborío (2), J. A. McInroy (1)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Centro de Investigaciones Agronómicas, Universidad de Costa Rica, San José, COSTA RICA
Phytopathology 100:S63

Fern distortion syndrome (FDS), a newly described disease of Leatherleaf fern (*Rumohra adiantiformis*), causes significant damage in ferneries of Costa Rica. The main aboveground symptoms of FDS are twisting and distortions of fronds, making fronds unmarketable. A recent report found an association of symptoms with increased endophytic rhizome populations of fluorescent pseudomonads (FPs), and constituted the first steps of Koch's postulates (demonstrating a constant association of the suspected causal agent with the disease and isolation of the agent). Here we report that strains of FPs isolated from rhizomes, roots, and petioles of ferns with FDS symptoms cause the typical symptoms of FDS in two greenhouse experiments conducted over a three-year period. In the first test, rhizomes of asymptomatic ferns were collected in the field and treated at the time of transplanting in the greenhouse with log 6 and log 8 cfu/ml with 6 groups of FPs isolated from symptomatic fern plants. In the second test, tissue culture plants were grown until rhizomes were present, which were treated similarly to the first experiment, and an additional 2 treatments were also used: log 6 and log 8 cfu/ml of FPs isolated from healthy-appearing ferns. Ferns treated with the FPs isolated from symptomatic ferns, but not those treated with FPs from asymptomatic plants, developed distortions typical of FDS, including twisting of the frond rachis, loss of triangular shape of frond, and reduced size of fronds.

Comparative genomics of the plant vascular wilt pathogens, *Verticillium dahliae* and *Verticllium albo-atrum*

S. J. KLOSTERMAN (1), K. V. Subbarao (2), S. Kang (3), P. Veronese (4), S. E. Gold (5), B. P. Thomma (6), Z. Chen (7), B. Henrissat (8), Y. Lee (9), J. Park (9), M. D. Garcia-Pedrajas (10), D. Barbara (11), A. Anchieta (1), R. de Jonge (6), P. Santhanam (6), K. Maruthachalam (2), Z. K. Atallah (2), S. Amyotte (12), Z. Paz (5), P. Inderbitzen (13), D. Heiman (7), S. Young (7), Q. Zeng (7), R. Engels (7), M. Koehrsen (7), J. Galagan (7), B. Birren (7), C. Cuomo (7), K. F. Dobinson (12), L. Ma (7)
(1) USDA ARS, Salinas, CA, U.S.A.; (2) UC Davis, Salinas, CA, U.S.A.; (3) Pennsylvania State University, University Park, PA, U.S.A.; (4) North Carolina State University, Raleigh, NC, U.S.A.; (5) University of Georgia, Athens, GA, U.S.A.; (6) Wageningen University and Research Center, Wageningen, NETHERLANDS; (7) Broad Institute, Cambridge, MA, U.S.A.; (8) Architecture et Fonction des Macromolecules Biologiques, CNRS, Universites Aix-Marseille, Marseille, FRANCE; (9) Department of Agricultural Biotechnology, Seoul National University, Seoul, KOREA; (10) Estacion Experimental La Mayora, CSIC, Malaga, SPAIN; (11) University of Warwick, Warwick, ENGLAND; (12) Agriculture and Agri-Food Canada, London, ON, CANADA; (13) UC Davis, Davis, CA, U.S.A.
Phytopathology 100:S64

Verticillium dahliae and *Verticillium albo-atrum* are plant pathogenic fungi that cause Verticillium wilts worldwide. The 7.5 X sequence of *V. dahliae* strain VdLs.17 and the 4 X sequence of *V. albo-atrum* strain VaMs.102 were generated and assembled at the Broad Institute using Sanger sequencing. A comparison of these genomes revealed a high level of synteny between these two *Verticillium* species, and led to the identification of a set of potential effector proteins. In particular, our study revealed higher numbers of pectinolytic enzymes in the *Verticillium* species than in other fungi, which may have direct implications in the ability of these pathogens to colonize a wide range of plant hosts. Additionally, we identified in the genome assembly of *V. dahliae* strain VdLs.17 four lineage-specific (LS) regions which are absent from VaMs.102. Certain gene families in the transposon-rich LS regions have undergone expansion, including transcription factors, ferric reductases, and phospholipases, which collectively may facilitate niche adaptation. Comparative analyses with another vascular wilt fungus, *Fusarium oxysporum*, revealed a conserved set of proteins that may have particular relevance for these vascular wilt fungi. These findings provide insight into the molecular determinants that underpin pathogenicity and niche adaptation in these vascular wilt fungi, and provide a foundation for functional genomics analyses.

Novel fungicide timings to target important turfgrass diseases in the upper Midwest

P. KOCH (1), J. Kerns (2)
(1) University of Wisconsin - Madison, Verona, WI, U.S.A.; (2) University of Wisconsin - Madison, Madison, WI, U.S.A.
Phytopathology 100:S64

The primary diseases of amenity turfgrass throughout the Upper Midwest are dollar spot, Microdochium patch and Typhula blight. Previous research has shown that fungicide applications made well before dollar spot symptom development delays symptom development and reduces overall fungicide usage when compared to a calendar-based program. Yet early season applications do not provide season-long control of dollar spot or target most other turfgrass diseases. This research examines novel fungicide application timings targeting dollar spot, Microdochium patch and Typhula blight in order to reduce fungicide expenditures while maximizing disease control. Applications of boscalid or a tank-mixture of iprodione and chlorothalonil were made in combinations of four different fungicide timings consisting of early fall, late fall, early spring, and late spring. Fungicide applications made at both the early and late spring timings provided the most effective control of dollar spot, though they did not control Microdochium patch and Typhula blight. Applications made at all four timings provided the best suppression of dollar spot, Microdochium patch, and Typhula blight. Although, dollar spot suppression only lasted until mid-July. Implementing an integrated program in mid-July would provide season-long dollar spot and snow mold control with as little as 4 or 5 fungicide applications.

Validation of commercially available ELISA kits for analyzing chlorothalonil and iprodione residues on creeping bentgrass using gas chromatography

P. KOCH (1), J. Kerns (2)
(1) University of Wisconsin, Verona, WI, U.S.A.; (2) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S64

Commercially available enzyme-linked immunosorbent assay (ELISA) kits (Horiba, Ltd) can determine concentrations of chlorothalonil and iprodione on fruits and grains quickly and accurately. To be used on turfgrasses, modifications to the ELISA protocol provided by Horiba, Ltd were required to accommodate smaller quantities of plant material collected from creeping bentgrass mowed at fairway height. The amount of tissue was reduced from 5 g to 0.2 g and the tissue was pulverized with a solvent for extraction of the fungicides with/within the plant tissue. Validation of the modified ELISA method was conducted in the fall of 2009 by comparing fungicide concentrations collected from ELISA and gas chromatography/flame ionized detection (GC/FID). This was accomplished by analyzing six samples of creeping bentgrass for fungicide concentration one hour following the application of chlorothalonil and iprodione using the altered ELISA method as well as GC/FID. Chlorothalonil was not accurately detected using the ELISA method, while measured levels of iprodione were consistently five times lower than what was detected with GC/FID. The ELISA method was further modified to be more consistent with the Horiba, Ltd protocol, and subsequent comparisons between ELISA and GC/FID were completed on April 14 and May 12. A reliable ELISA method for the detection of fungicides in turfgrass can be used to provide insight into the role that fungicides play in disease control.

Search for fungi as potential biological control agents of *Salsola tragus*

T. M. KOLOMIETS (1), D. K. Berner (2), Z. M. Mukhina (3), A. V. Alexandrova (4), O. O. Skatenok (1)
(1) All Russian Research Institute of Phytopathology, Moscow region, Bolshie Vyazemi, RUSSIA; (2) Foreign Disease-Weed Science Research Unit, USDA, ARS, Frederick, MD, U.S.A.; (3) All Russian Scientific Research Institute of Rice, Krasnodar, Belozerniy, RUSSIA; (4) M.J. Lomonosov's Moscow State University, Moscow, RUSSIA
Phytopathology 100:S64

Weeds of genus *Salsola* (family *Chenopodiaceae*) are widespread inhabitants of many vegetative communities in damp territories and cultivated fields. Plants of this genus are resistant to drought and are salt tolerant. Eurasia is the natural habitat for *Salsola*. *Salsola tragus* is a widespread invasive weed problem in the U.S.A. and is found in most states. In Russia *Salsola tragus* L. is widespread in the Caucasuses, the southern areas of the European part of Russia, in Siberia and in Central Asia. This weed infests grain fields, pastures, rangelands, and roadsides. Because *S. tragus* is found mostly on low-value land, control with herbicides is not economical. Thus, there is a necessity to develop biological means of control. For use in classical biological control. a complex of fungi were identified on *Salsola tragus*, These included the obligate pathogen *Uromyces salsolae* Reich *Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc., *Colletotrichum coccodes*, *Phomopsis oblonga*, *Alternaria alternata* (Fr.) Keissl., *Epicoccum nigrum* Link, *Pythium* sp., Sordaria sp, *Arthrinium arundinis* (Corda) Dyko and B. Sutton, *Arthrinium phaeospermum* (Corda) M.B. Ellis., *Bipilaris sorokiniana*, *Phoma herbarum* Westend., *Ascochyta caulina* (P. Karst.) Aa et Kesteren, *Papulaspora sepedonioides* Preuss. *Bionectria* sp., *Nigrospora oryzae* (Berk. And Broome) Petch., *Nigrospora sphaerica*, *Stemphylium herbarum*, *Fusarium fujikuroi* Nirenberg., and *Aspergillus versicolor* (Vuill.) Tirab. Of these fungi, *Uromyces salsolae* Reich., *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Phomopsis oblonga* (*Diaporthe eres* Nitschke) have great potential for classical biological control of *Salsola tragus*.

Long-term survival of *Xanthomonas fragariae* in infected strawberry leaf tissue

M. KONG (1)
(1) Driscoll Strawberry Assoc Inc, Watsonville, CA, U.S.A.
Phytopathology 100:S64

Xanthomonas fragariae is the pathogen that causes Angular Leaf Spot of Strawberry, a very common and sometimes quarantine-related disease in many countries. It is a common cause of disruptions in the movement of strawberry plants across borders. To eliminate or reduce *X. fragariae* contamination levels, short-term survival of *X. fragariae* under different temperatures has been the subject of a number of studies. At Driscoll's, we completed a study of long-term *X. fragariae* survival in infected leaf material under relatively favorable storage conditions. During April 1988, we prepared *X. fragariae* by surface sterilizing strawberry leaves with Angular Leaf Spot symptoms. Leaf spots were cut out from symptomatic leaves and air dried in a laminar flow hood then stored in tape-sealed Petri dishes in a refrigerator set at 5°C. Actual temperatures may have fluctuated from 0 to 15°C for short periods. We have tested a few of the leaf spots every few years for viability. This was done by plating the bacterial suspension that oozed out from the leaf spots and incubating this suspension at room temperature. During March 2009, after almost 21 years of storage we were still able to recover living *X. fragariae*. This recovered *X. fragariae* was still virulent and Angular Leaf Spot symptoms arose on susceptible plants that were inoculated.

Genome-wide functional analysis of bZIP transcription factors in the rice blast fungus

S. KONG (1), S. Park (1), K. Jung (1), Y. Lee (1)
(1) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, SOUTH KOREA
Phytopathology 100:S65

Basic leucine zipper (bZIP) proteins are major eukaryotic transcription factors (TFs). Since fungal lifestyles depends on adaptability in environments, it is pivotal to elucidate the transcriptional programs operating under different conditions such as physical and chemical stresses, and host-dependent constraints. In this study, bZIP TFs in *Magnaporthe oryzae* (*MobZIP*s) were systemically characterized using phylogenetic, expression profiling and gene deletion analyses. bZIP TF sequences from 23 fungal species were identified and their phylogenetic relationship was analyzed. In total, 9 out of 15 groups include at least one functional orthologs. Quantitative RT-PCR analysis for *MobZIP* genes on 32 different conditions showed dynamic expression profiles, suggesting their involvement in various stress responses and during pathogenesis. To link phylogenetic and expression data to phenotypes, gene deletion was conducted for 2 *Magnaporthe*-specific (MGG_02865.6, MGG_07305.6) and 2 having orthologs (MGG_01990.6, MGG_02006.6). Phenotype changes, however, were not detectable on these mutants for mycelia growth in several stress conditions and pathogenicity on susceptible rice plants. These results suggest that fungal bZIP TFs within the same clade may have different roles depend on the lifestyles, and expression pattern does not always directly reflect its roles on phenotypes.

Dose-response relationship in UV-C induced disease resistance and phytoalexin accumulation in stored carrots

N. KOUASSI (1), R. Corcuff (1), R. Tweddell (2), J. Arul (1)
(1) Department of Food Science and Nutrition and Horticultural Research Center, Universite Laval, Quebec, QC, CANADA; (2) Horticultural Research Center, Université Laval, Quebec, QC, CANADA
Phytopathology 100:S65

Major limitation in long-term storage of carrots is the diseases caused by *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Intensification of the natural defenses of the plant can be an attractive approach to protect stored crops without resorting to chemicals. We have shown previously that UV-C induces disease resistance in carrots. The objective of this work was to determine the dose-response relationship in UV-C induced disease resistance and 6-methoxymellein (6-MM) accumulation. Carrots (cv. Sun255) were exposed to UV doses ranging from 0.0 - 10.8 kJ.m^{-2}, and were stored at 4°C under high relative humidity. After 14 days of storage, treated carrots were sampled to assay 6-MM in the peel. After 28 days, treated carrots were inoculated with 3-day old mycelial plugs of either *B. cinerea* or *S. sclerotiorum* and the severity of infection assessed 21 days after inoculation. The dose-response relationship for disease resistance to *B. cinerea* or *S. sclerotiorum* as well as 6-MM accumulation in carrots was biphasic. The severity of diseases progressively decreased compared with control roots up to a dose of about 5.4 kJ.m^{-2}. But beyond that dose, disease severity increased. Maximum accumulation of 6-MM was also observed in carrots treated with the dose of 5.4 kJ.m^{-2}. It was concluded that beneficial or hormetic UV dose for improving disease resistance of carrots was 5.4 kJ.m^{-2} and that the level of 6-MM present in carrots before inoculation was a significant factor contributing to resistance.

Response of U.S. bottle gourd (*Lagenaria siceraria*) plant introductions (PI) to crown rot caused by *Phytophthora capsici*

C. S. KOUSIK (1), J. A. Thies (2)
(1) USDA ARS, Charleston, SC, U.S.A.; (2) U.S. Vegetable Laboratory, USDA ARS, Charleston, SC, U.S.A.
Phytopathology 100:S65

Phytophthora capsici can cause severe damage to cucurbit crops grown in open fields in the southeast regions of U.S.A. In recent years there has been a growing interest in the U.S.A. in grafting watermelon plants onto various cucurbit rootstocks including bottle gourds for managing soil borne diseases. We evaluated over 200 U.S. Plant Introductions (PI) of bottle gourd for resistance to crown rot caused by *P. capsici* in the greenhouse by inoculating four week old seedlings with a zoospore suspension (10^4/ml/plant). Plants of watermelon variety 'Mickey Lee' were used as the susceptible check. This trial was conducted twice. Plants were rated on a 1–9 scale of increasing disease severity where 1 = no symptoms to 9 = plants dead. All the plants of 'Mickey Lee' were dead within 2–3 weeks after inoculation. Eleven (5.2%) of the PIs tested were resistant to *P. capsici*. Of these 11 four were resistant and 7 were moderately resistant. Variability in the level of resistance of individual plants within PIs also was observed. Based on the two evaluations, 42 PIs were evaluated again and rated four times over 50 days after inoculation. Disease development was significantly (P = 0.05) slower on PI 271352, PI 497351, PI491278 and PI 487482 compared to checks. These PIs can be considered as potential sources of resistance to *P. capsici*. Single plant selections from resistant PIs are being made for use in our rootstock breeding program.

Evaluation of wheat world genetic collections to harmful pathogens

E. D. Kovalenko (1), T. M. Kolomiets (1), M. I. KISELEVA (1), I. F. Lapochkina (2), I. B. Ablova (3), Z. N. Khudokormova (3), H. Bockelman (4)
(1) All Russian Research Institute of Phytopathology, Moscow region, Bolshie Vyazemi, RUSSIA; (2) Agriculture Research Institute of Non Chernozem Zone, RUSSIA; (3) Krasnodar Agriculture Research Institute named after P.P. Lukyanenko, RUSSIA; (4) USDA-ARS Germplasm Resources Information Network (GRIN), Aberdeen, ID, U.S.A.
Phytopathology 100:S65

Puccinia triticina, *Septoria* sp. and powdery mildew are the most harmful diseases of wheat in Russia. Nine hundred and twenty-two winter and spring wheat accessions from NSGC, ARIPI, ARINCZ, and CIMMYT were evaluated for leaf rust, septoriosis and powdery mildew in the infection nurseries of Central region: All Russian Research Institute of Phytopathology (ARRIP), Agriculture Research Institute of Non Chernozem Zone (ARINCZ), and North Caucasian region: Krasnodar Agriculture Research Institute (KARI). The leaf rust pathotypes and Septoria strains characterized genetic diversity, high virulence and aggressive were selected for creation artificial infectious backgrounds. Wheat cultivars were evaluated for resistance to powdery mildew in natural infection environments. The main resistance types to septoria and leaf rust in winter and spring wheats were identified based on field and laboratory data. As a result of a study of partial resistance components in field nursery (AUDPC) and in an artificial climate chamber (latent period, pustule/spot density, pustule/spot size) the genotypes with high level of partial resistance to leaf rust and Septoria were identified. The most of accessions with race-specific and partial resistance to leaf rust was found out among synthetic lines of wheat from USDA-ARS and spring wheat cultivars from CIMMYT. Resistant and moderately resistant to septoriosis cultivars and group resistant to leaf rust, Septoria and powdery mildew wheat samples were revealed. Wheat cultivars with complex resistance, partial resistance and combined different resistance types are represented the greatest interest for breeding. The juvenile genes of resistance to leaf rust were identified for more than 100 wheat cultivars by the phytopathologic testing and the STS and SSR-markers. Resistant wheat cultivars from National Small Grains Collection (NSGC) with complex of economically valuable features for quality improvement of wheat cultivars in Central region of Russia were selected. The following hybrid lines of spring wheat: PI 520375 (Mexico), PI 519425, PI 520528 (U.S.A., N. Dakota) and winter wheat: PI 564430, PI 564291 (Bulgaria), PI 422224, PI 547264, PI 547262 (England), PI 604222 (U.S.A., KS) and PI 447407 (China), can be recommended for breeding program.

Field efficacy of *Bacillus subtilis* MBI 600 (Integral^{R}) for managing rice sheath blight caused by *Rhizoctonia solani*

V. KRISHNA KUMAR (1), M. Reddy (1), J. W. Kloepper (1), K. S. Lawrence (1), S. Krishnam Raju (2), D. Groth (3), M. Miller (1)
(1) Auburn University, Auburn, AL, U.S.A.; (2) Andhra Pradesh Rice Research Institute, Acharya NG Ranga Agricultural University, Maruteru, INDIA; (3) Rice Research Station, Louisiana State University Ag Center, Rayne, LA, U.S.A.
Phytopathology 100:S65

Rice sheath blight (ShB) caused by Rhizoctonia solani causes significant yield losses in all rice producing areas of the world. Nursery and field trials were conducted to assess the PGPR product Integral in India during 2009 against ShB. Integral was applied as seed treatment (ST), seedling root dip (SD) and foliar spray (FS) at concentrations of log 8 - 9 cfu per ml. Seedling growth parameters and ShB severity were measured by adopting Highest Relative Lesion Height (HRLH) at 90 days after transplanting. Seed bacterization with Integral resulted in enhanced root (9.3 to 14 cm) and shoot lengths (37 to 45 cm) over the control (8.4 and 36 cm respectively) in nursery. On a transplanted crop in the field, ShB severity was significantly lower when Integral was applied as ST + SD + FS at 2.2×10^9 cfu ml^{-1} (19.2 to 26.5), followed by at 2.2×10^8 cfu ml^{-1} (24.5 to 29.4) compared to control (56.2 to 69.7). The ShB severity in carbendazim treated plants ranged from 16.8 to 19.8. In addition, the tiller production/plant was significantly higher in Integral treated plots at 2.2×10^9 cfu ml^{-1} (12.3 to 12.9) compared to the control (10.0 to 10.5). Highest grain yields were recorded in Integral treated plots at 2.2×10^9 cfu ml^{-1} (5922 to 6207 kg/ha) compared to the control (3925 to 4199 kg/ha). Overall, Integral significantly reduced the ShB severity, and increased seedling vigor and grain yields in rice under field conditions.

Multi-scale spatial heterogeneity in disease incidence of Fusarium head blight of wheat

A. B. KRISS (1), P. A. Paul (1), L. V. Madden (1)
(1) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S65

A survey for the incidence of Fusarium head blight (caused by Fusarium graminearum) was conducted in Ohio during the 2002 through 2009 growing seasons. Sampling was conducted by counting the number of diseased and healthy wheat spikes per 0.3 m of row at ten sites (about 30 m apart) in each of 67-159 sampled fields in each of 12-31 sampled counties per year. Incidence was then determined as the proportion of diseased spikes at each site. Spatial heterogeneity of incidence among counties, fields within counties, and sites within fields was characterized by fitting a generalized linear mixed model (GLMM) to the data, using a complementary log-log link function, with the assumption that the disease status of spikes was binomially distributed conditional on the effects of county, field and site. The marginal model for an individual field corresponds to an overdispersed discrete distribution. Based on the estimated variance terms, there was highly significant spatial heterogeneity among counties each year, and also among fields within counties; magnitude of the estimated variances was similar for counties and fields. The degree of heterogeneity varied significantly among years. The lowest level of heterogeneity was among sites within fields, and the site variance was not significantly greater than 0 in three of the eight years. Based on the among-site variances, the intra-cluster correlation of disease status of spikes within sites was low in most fields.

Role of El Niño-Southern Oscillation and atmospheric teleconnection patterns on variability of Fusarium head blight in Ohio

A. B. KRISS (1), P. A. Paul (1), L. V. Madden (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S66

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a sporadic disease that is dependent, at least in part, on weather and climatic conditions. To investigate the effect of climate on FHB, we used cross-spectral analysis which is a tool used to partition the variance of time-series data into temporal scales or periods. The time-series investigated were the Southern Oscillation Index (SOI), which is a measure of the El Niño-Southern Oscillation (ENSO), three atmospheric teleconnections [Arctic Oscillation (AO), Pacific/North American (PNA) pattern, North Atlantic Oscillation (NAO)] and Fusarium head blight rating in Ohio from 1965 to 2009. Mean index values for December to February and March to May were used because winter/spring conditions are important for *F. graminearum* survival and development, thereby affecting disease. FHB and winter/spring SOI were coherent at a periodicity of 5 years (K2 = 0.75/0.70). The phase relationships showed that the SOI series lead FHB by 0.95 to 1.2 years. Consistent with other studies, the most significant teleconnection related contributions were for the winter months. FHB and winter PNA were coherent at a periodicity of 5.6 years (K2 = 0.66), FHB and winter AO were coherent at a periodicity of 2.3 years (K2 = 0.65), and FHB and NAO were coherent at a periodicity of 4.5 to 5 years (K2 = 0.66).

Simple, rapid, and specific DNA-based diagnostics for detection of the bacterial wilt pathogen *Ralstonia solanacearum* Race 3 Biovar 2

R. KUBOTA (1), M. A. Schell (2), G. D. Peckham (1), A. M. Alvarez (1), C. Allen (3), D. M. Jenkins (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.; (3) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S66

Simple, rapid, and specific diagnostic tools are urgently needed to discriminate the quarantine pathogen *Ralstonia solanacearum* (Rs) Race 3 Biovar 2 (R3B2) from other populations of Rs that lack the adaptation to cause bacterial wilt disease in temperate regions. Isothermal DNA amplification techniques, such as Loop-mediated isothermal AMPlification (LAMP), are suitable for rapid detection of bacteria with simple devices due to the ability to amplify DNA with high specificity, efficiency, and speed at a constant temperature. Three R3B2-specific LAMP primer sets were designed by targeting previously identified unique R3B2 DNA sequences, and each LAMP primer set was evaluated for its specificity, reaction time, and process simplicity. The specificity of LAMP was assessed against a comprehensive collection of 264 geographically diverse Rs-complex strains and 4 non-Rs bacteria, and of these only a single Rs strain (UW348) failed to react with two of the LAMP primer sets. The speed of LAMP reactions was monitored by observing the fluorescence of a commercially available intercalating dye, and the optimal reaction was completed within 20 min. The potential simplicity of LAMP procedure was evaluated by omitting the denaturing and cooling steps, and two of the LAMP primer sets amplified under the simplified procedure. These results illustrate a promising alternative for the development of simple, rapid, and specific diagnostics for Rs subpopulations.

Comparison of the rainfastness of Revus® and Forum® on lettuce for control of downy mildew (*Bremia lactucae*)

P. KUHN (1), A. Hert (1), G. Olaya (1), L. Mantoe (2), G. Knauf-Beiter (2)
(1) Syngenta Crop Protection, Vero Beach, FL, U.S.A.; (2) Syngenta Crop Protection, Stein, SWITZERLAND
Phytopathology 100:S66

Resistance to washoff by rain or overhead irrigation is an important characteristic of crop protection products including fungicides. We compared this property for the fungicides Revus (Syngenta) and Forum (BASF) applied to lettuce for control of downy mildew (*B. lactucae*). Fungicides were applied at various rates with or without an adjuvant, followed by artificial rainfall applied at different times after treatment via either a flat fan nozzle in a cabinet spray chamber or from a purpose-built rain simulator. Fungicide-treated plants that received no artificial rain, and untreated, inoculated plants were included as controls. Revus at all rates provided excellent disease control. In addition, efficacy from Revus was not diminished by water or simulated rain even when applied at only 30 or 45 mins after fungicide treatment. These observations are consistent with Revus having a very high degree of rainfastness. Irrespective of the washoff régime, Forum was consistently less effective than Revus. Furthermore, the level of disease control from Forum was markedly reduced after simulated rain, indicating poor rainfastness over the time intervals evaluated.

Gene expressions of effectors in downy mildew of lima bean pathogen, *Phytophthora phaseoli*

S. G. KUNJETI (1), N. M. Donofrio (1), A. G. Marsh (1), B. C. Meyers (1), T. A. Evans (1)
(1) University of Delaware, Newark, DE, U.S.A.
Phytopathology 100:S66

Lima bean is an important legume crop for the state of Delaware. This crop is susceptible to two oomycete pathogens, *Phytophthora phaseoli* and *P. capsici*, causing downy mildew and pod blight of lima bean, respectively. In this study we have identified several genes in *P. phaseoli* orthologous to effector genes in *P. infestans*, a close relative of *P. phaseoli*. To initially identify these effector genes, the Illumina next-generation sequencing platform was used for profiling the transcripts of plant-grown and plate-grown *P. phaseoli*. Full-length sequence analysis of three RxLR effector genes showed 97, 94, and 91 percent identity to amino acid sequences of PITG_17063, PITG_15039, and PITG_04074 genes of *P. infestans*, respectively. In addition, two elicitors showed 96 percent identity to the amino acid sequences of the INF1 and INF4 genes of *P. infestans*. The above five effector genes were validated by performing in-planta RT-PCR. The two elicitors INF1 and INF4 were over-expressed in plant-grown when compared to plate-grown tissue in both *P. phaseoli* and *P. infestans* whereas only INF1 was expressed in plant-grown *P. capsici*. A phylogenetic analysis of all five effector genes from other oomycete pathogens confirmed a close relationship of *P. phaseoli* and *P. infestans* for all the corresponding effector genes. Currently, we are performing functional characterization of these effector genes, which will help us gain a better understanding of this pathosystem and will serve as a basis for future research.

Towards solving 'inconclusive' quantitative PCR for the presence of Huanglongbing (HLB) in orange jasmine leaf samples in Texas

M. KUNTA (1), J. V. da Graça (1), M. Sétamou (1), M. Skaria (1)
(1) Texas A&M University-Kingsville Citrus Center, Weslaco, TX, U.S.A.
Phytopathology 100:S66

As part of an ongoing survey for citrus huanglongbing (HLB) in Texas, quantitative PCR (qPCR) tests for the casual bacteria, '*Candidatus*' Liberibacter spp. in citrus and non-citrus species such as orange jasmine and the vector, the Asian citrus psyllid (ACP) were conducted. Although all citrus and ACP samples were tested negative so far for *Ca*. Liberibacter asiaticus, 44 orange jasmine samples yielded Ct values above 32. Some of the Ct values were confirmed by the Plant Protection and Quarantine Molecular Diagnostic Lab, Beltsville, MD, and designated as 'inconclusive.' The samples that yielded inconclusive were resampled by collecting leaves and psyllids from different parts of the plant. A total of 440 samples were collected from 55 plants (8/plant). High Ct values were obtained from 24 of these plants. More inconclusive results were obtained in the samples collected from the south side of the plants. All the psyllid samples were tested negative for *Ca*. Liberibacter asiaticus. Nested PCR assays on the DNA extracts producing high qPCR Ct values resulted in amplification of plant chloroplast 16S rDNA and non-specific amplicons. Since orange jasmine is an alternative host of the HLB bacterium, a traditional approach is being used to obtain 16S rDNA sequences of any other bacteria which may be resident in these orange jasmine plants.

Five years of monitoring foliar diseases of soybean in Minnesota

J. E. KURLE (1), D. K. Malvick (2), C. M. Floyd (2), G. M. Anderson (2)
(1) University of MN Department of Plant Pathology, Minneapolis, MN, U.S.A.; (2) University of MN Department of Plant Pathology, St. Paul, MN, U.S.A.
Phytopathology 100:S66

Several important foliar diseases occur on soybean, and soybean rust (SBR) caused by *Phakopsora pachyrhizi* can be one of the most destructive to crop yields. Although SBR has been detected in the southern and central U.S., it has not been found in Minnesota (MN). For SBR to occur in MN, urediniospores must be transported to MN and be deposited when environmental conditions favor infection and disease development. From 2005 through 2009, up to 36 soybean sentinel plots were established each year in commercial soybean fields or at University of Minnesota research fields as part of a nationwide USDA Pest Information Platform for Extension and Education. A minimum of 100 soybean trifoliate leaves from each plot were analyzed weekly during the growing seasons to determine if SBR was present. The leaf samples were incubated and then analyzed microscopically for SBR and other foliar diseases. Spore deposition samplers were also placed in sentinel plots during the growing seasons to monitor for SBR urediniospores. Filters from spore samplers were collected weekly and were analyzed for urediniospores using a nested PCR assay. Soybean rust spores were detected in 2005, 2006, and 2007. Soybean rust was not found during the five years of monitoring in MN, however, the sentinel plot effort provided a comprehensive picture of other foliar diseases of soybean, including brown spot, downy mildew, and bacterial blight.

Determining the effects of foliar and heading diseases on soft red winter wheat (*Triticum aestivum*) yield in Wisconsin

K. LACKERMANN (1), J. Gaska (1), M. Martinka (1), S. Conley (1), P. Esker (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S67

Foliar and heading diseases both reduce grain yield, but they occur at different times during the growing season and can be managed independently. To improve understanding of the effect of foliar and heading diseases on wheat grain yield, data were collected from winter wheat variety trials at four locations (Arlington, Chilton, Janesville, Lancaster). Fifty-eight winter wheat cultivars were planted in a randomized complete block design with four replications. Focus was on four foliar diseases (powdery mildew (PM), Septoria leaf blotch (SLB), leaf rust (LR), and stripe rust (SR)) and one heading disease (Fusarium head blight, FHB). Assessments were made four times for foliar diseases and one time for FHB. For each foliar disease assessment, a weighted disease severity value (WS) was calculated for the upper four leaves. At Zadoks 60 (flowering), the WS ranged from 0 to 418 (PM), 0 to 421 (SLB), 0 to 293 (LR), and 0 to 121 (SR). FHB severity at Zadoks 85 (soft dough) ranged from 0 to 11% with the highest severity noted at Lancaster. Grain yields ranged from 2.5 to 7.5 MT/ha. The lowest mean yield (4.2 MT/ha) was observed at Janesville, which had low levels of FHB (0 to 3.9%), but higher levels of SLB (0 to 421) and LR (0 to 213). There was high variability in cultivar response to disease and subsequent grain yield. In 2009 foliar diseases may have caused more yield loss than FHB due to the low incidence of FHB.

Effect of environment, cultivar, and disease on soft red winter wheat (*Triticum aestivum*) production in Wisconsin

K. LACKERMANN (1), J. Gaska (1), M. Martinka (1), S. Conley (1), P. Esker (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S67

Little is known about the impact of foliar diseases on wheat yield in Wisconsin. Our objective was to compare the yield and disease incidence of wheat cultivars at several environments in WI. Fifty eight wheat cultivars were planted in a randomized complete block design at three locations (Arlington, Chilton, Lancaster). At the fourth location (Janesville), the design was a split-plot with a Zadoks 45 foliar fungicide application at the whole plot level. Disease assessments were made at four times during the growing season for powdery mildew (PM), Septoria leaf blotch (SLB), and leaf rust (LR). A mixed model was used to study the effects of environment, cultivar, and disease severity on grain yield, and the effect of environment and cultivar on disease. Effects were significant at $P = 0.05$. Overall, SLB and LR were the most prevalent diseases and were uniformly distributed across environments. PM was most prevalent at Arlington and Chilton. Yield was affected by location, cultivar, SLB, LR, and the location × cultivar interaction. SLB and LR were affected by cultivar while PM was affected by location, cultivar, and the location × cultivar interaction. At Janesville, the analyses for yield and for disease revealed an effect of cultivar and PM on yield and an effect of cultivar on SLB, LR, and PM, but no effect of fungicide on yield or disease. Our results suggest that wheat growers should use cultivar selection to manage disease incidence and maintain high yields.

***Agrobacterium*-mediated transformation of *Fusarium oxysporum* f. sp. *gladioli* to study pathogenesis in gladiolus**

D. K. LAKSHMAN (1), R. Pandey (2), K. K. Kamo (3), A. Mitra (4)
(1) Sustainable Agricultural Systems Laboratory, USDA-ARS, Beltsville, MD, U.S.A.; (2) University of Lucknow, Lucknow, INDIA; (3) Floral and Nursery Plants Research Unit, USDA-ARS, Beltsville, MD, U.S.A.; (4) Dept. of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE, U.S.A.
Phytopathology 100:S67

Fusarium rot caused by *F. oxysporum* f. sp. *gladioli* (FOG) is one of the most serious diseases of gladiolus, both in the field and in storage. Traditionally, the pathogen had been controlled using methyl bromide soil fumigations that are now banned, hot water treatments and to a limited extent, using tolerant cultivars; necessitating further studies into host-pathogen interactions of the pathogen on gladiolus. In this vein, we have developed an *Agrobacterium*-mediated FOG transformation system using the pBGgHg vector (supplied by Prof. S. Kang, Penn State) in AGL1 strain of *A. tumefaciens*. Hygromycin (100 µg/ml) resistant (Hyg^R) colonies were observed only when acetosyringone (AS) was added to the co-cultivation medium. Also, the efficiency of transformation increased when the *Agrobacterium* culture was not pre-induced with AS before the cocultivation step and when cellophane instead of Hybond-N+ membrane was used during cocultivation in medium containing AS. Transformed isolates were selected by at least four serial transfers in medium containing Hyg. The transformed mycelia expressed green fluorescence which was not observed with non-transformed isolates. PCR with Hyg-specific primers were positive from Hyg^R isolates and not from untransformed isolates. Transformed FOG isolates will be evaluated for pathogenicity and behavior in transgenic gladiolus expressing chitinase genes.

Rose rosette and Redbud yellow ringspot are caused by two new emaraviruses

A. G. LANEY (1), R. Gergerich (1), K. Keller (2), R. Martin (2), I. Tzanetakis (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S67

Rose rosette (RR) was first described in Canada in the 1940s and Redbud yellow ringspot (RYRS) in Arkansas in the 1970s. Since then, the only major breakthroughs on the etiology of RR or RYRS was the discovery of double membrane-bound bodies in symptomatic plants of both diseases and evidence that the RR agent is transmitted by eriophyid mites. RR, a widespread disease east of the Mississippi River and a major threat to the ornamental industry, is usually associated with witches' broom, lateral shoot elongation, and malformation of flowers and leaves, culminating in plant death. RYRS, a disease with unknown geographic distribution, causes chlorotic ringspots, oak-leaf, and vein chlorosis in mature leaves. We have acquired data suggesting that two new negative-stranded RNA viruses, members of the newly established genus *Emaravirus*, and provisionally named Rose rosette-associated virus (RRaV) and Redbud yellow ringspot-associated virus (RYRSaV), are associated with RR and RYRS respectively. Detection protocols have been developed and used to survey symptomatic roses and redbuds for the respective viruses. Both viruses were found in almost all diseased samples. Potential field alternative hosts were surveyed and several herbaceous hosts were inoculated mechanically and by grafting. Transmission studies for RYRSaV using an *Aceria* species eriophyid mite are under way.

Management of Verticillium wilt of potato with disease-suppressive crop rotations

R. P. LARKIN (1), W. Honeycutt (1), M. Olanya (1)
(1) USDA ARS, Orono, ME, U.S.A.
Phytopathology 100:S67

The ability of potential disease-suppressive rotation crops to reduce potato disease problems and increase crop productivity in a field severely infested with Verticillium wilt was evaluated over three field seasons in Maine. Rotation treatments consisted of 1) a high glucosinolate mustard blend ('Caliente 119'), a mixture of white mustard and oriental mustard with known biofumigation potential, and 2) a sorghum-sudangrass hybrid, both grown as green manures. These rotations were compared with a standard barley rotation and a barley rotation followed by chemical fumigation with Metham sodium as controls. Both green manure rotations significantly reduced wilt in the subsequent potato crop compared to the barley control, with average reductions of 25 and 18%, respectively, but were not as effective as chemical fumigation (35% reduction). Mustard blend also reduced black scurf and common scab better than the other rotations. Mustard blend and chemical fumigation increased tuber yield relative to the barley control by 12 and 18%, respectively. However, by the second rotation cycle, disease levels were high in all rotations, and only chemical fumigation substantially reduced disease (by 35%). Rotations also had significant effects on soil microbiology and pathogen inoculum levels. This research indicates the potential for using disease-suppressive rotations for managing Verticillium wilt, but also demonstrates the limitations of 2-yr rotations regarding the build-up of soilborne diseases over time.

First report of *Cucurbit leaf crumple virus* in snap bean in Georgia
R. LARSEN (1)
(1) USDA ARS, Prosser, WA, U.S.A.
Phytopathology 100:S68

During October and November, 2009, a large commercial field planted with snap bean (*Phaseolus vulgaris*) variety 'Sea Biscuit' in Tift County, GA was observed exhibiting virus-like foliar symptoms consisting of interveinal chlorosis and vein greening, blistering, rugosity and malformation. Pods were curled, misshapen, and unmarketable. Symptoms were associated with the presence of *Bemesia tabaci* and infection rate in the field was estimated at >75%. Total nucleic acid was extracted from symptomatic leaf samples, and then amplicons generated by PCR using a set of degenerate primers specific for begomovirus coat protein (AV1 gene) were cloned and sequenced. The nucleotide and deduced amino acid sequences of the 533 bp fragment were 97% and 98% identical, respectively, with the Arizona isolate of *Cucurbit leaf crumple virus* (CuLCrV; a.k.a. Cucurbit leaf curl virus). Virus-like symptoms were observed in an adjacent field on variety 'Eliminator' where whiteflies were also present but the disease was determined not to be caused by CuLCrV. Hence, 'Eliminator' may be a potential source of resistance to the virus. CuLCrV has been restricted to cucurbits in California, Arizona and Florida until more recently when it was detected in fresh market beans in southwest Florida. This is the first report of CuLCrV in snap bean in Georgia. The increased area of detection suggests that the virus may pose a threat to common bean fields in other regions in the U.S. where *B. tabacci* are present.

Detection of *Pyrenophora teres* in infested plant tissues by PCR
R. T. LARTEY (1), T. Caesar-TonThat (1), A. J. Caesar (1), S. Hanson (1), U. M. Sainju (1)
(1) USDA ARS, Sidney, MT, U.S.A.
Phytopathology 100:S68

Net blotch of barley, a commonly occurring foliar disease is caused by *Pyrenophora teres* Drechs. The disease is characterized by small circular elliptical spots which enlarge to the typical narrow netlike pattern. Lesions in mature plants appear similar to spot blotch of *Cochliobolus sativus*, both of which occur extensively in the Northern Great Plains of the U.S. Conidia and ascospores from infested straw are probably the most important sources of primary inoculum. To speed up detection and identification of *P. teres* in infected hosts, a rapid PCR technique using Extract-N-Amp Plant PCR Kit (Sigma-Aldrich) was developed. Bypassing the standard DNA extraction, leaf disks from diseased tissues, uninfected barley leaves and *P. teres* pure culture controls were first homogenized in extraction solution and diluted with the solution provided in the kit. Aliquots of the homogenate were added to PCR reaction and subjected to amplification using the newly developed PTACTIN, a *P. teres* actin and ITS primers. Sizes of amplicons from diseases leaves which were resolved on agarose gel, correlated with amplicon from the control pure cultures. The amplicons were purified from the gel and sequenced. Sequences alignment confirmed the detection of *P. teres*. The technique will enhance rapid detection of *P. teres* in diseased barley and alternate hosts, including asymptomatic plants as well as verification of efficacy of management of net blotch.

Inferring evolutionary relationships of species in the *Phytophthora* Ic clade using nuclear and mitochondrial genes
E. S. LASSITER (1), C. Russ (2), C. Nusbaum (2), Q. Zeng (2), C. Hu (3), J. Thorne (1), J. B. Ristaino (3)
(1) Dept. of Genetics, North Carolina State University, Raleigh, NC, U.S.A.; (2) Broad Institute, Cambridge; (3) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S68

Phytophthora infestans the causative agent of potato and tomato late blight is an important pathogen worldwide and caused the Irish potato famine of the 1840's. Two sisters species of *P. infestans* in the Ic clade, *P. andina* and *P. mirabilis* have been described in Ecuador and Mexico, respectively. Coalescent analysis revealed that *P. andina* and *P. infestans* share a common ancestor in Ecuador on wild Solanum species. We have conducted Bayesian analysis of several nuclear genes (beta-tubulin, elongation factor Iα, RAS, and intron 1 of RAS) in isolates from Mexico and Ecuador and document the shared evolutionary history of the three species, which cluster together and are distinct from *P. phaseoli* and *P. ipomoeae*. Multiple heterozygous sites are shared among the three species. This is particularly interesting in the light of suggestion that *P. andina* may be a hybrid of *P. infestans* and *P. mirabilis*. We have also sequenced, annotated and mapped the entire mitochondrial genomes of *P. andina*, *P. mirabilis*, *P. ipomoeae* and *P. phaseoli* and compared then to the mitochondrial genome of *P. infestans*. We will report results of the Bayesian, coalescent and migration analysis using both the nuclear and whole mitochondrial genomes of these species and resolve the evolutionary histories of species of this clade.

LysM receptor-like kinase 1-mediated chitin signaling and fungal resistance in Arabidopsis
M. H. LE (1), X. Zhang (1), G. Stacey (1)
(1) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S68

Chitin, a polymer of β-1,4-N-acetyl glucosamine is an important component of the fungal cell wall. Chitin is one of the best studied pathogen-associated molecular patterns (PAMPs) and is capable of eliciting basal defense responses against fungal pathogens. Recently, the Arabidopsis receptor for chitin was identified, theLysM-containing receptor-like kinase 1 (LysM RLK1 (LYK1)/CERK1). However, little is known about the molecular basis of chitin perception and how chitin elicitation is translated into a cellular signal. To gain insight into chitin-elicited defense responses, we conducted a yeast two-hybrid based screen using the intracellular kinase domain of AtLYK1 as the bait. We identified 54 putative LYK1-interactors after screening 4.5 × 106 transformants. The annotations of these putative interactors identify them as both transmembrane and intracellular proteins, including transcription factors and kinases. T-DNA insertions in some of these genes resulted in a decrease in the production of reactive oxygen species after chitin treatment and increased susceptibility to pathogens, including the bacteria *Pseudomonas syringae* and the fungus *Alternaria brassicicola*. Therefore, these interactors may function as positive regulators in the chitin signaling pathway. We are currently working on further confirmation of the protein interactions detected by yeast two hybrid. This study will contribute to our understanding of chitin-elicited defense responses and to comparative studies of PAMP action.

Correlation of *Erwinia amylovora* virulence characteristics to disease severity in fire blight in apple trees and seedlings
S. A. LEE (1), H. K. Ngugi (2), T. W. McNellis (1)
(1) Dept. Plant Pathology, Penn State University, University Park, PA, U.S.A.; (2) Dept. Plant Pathology, Fruit Research and Extension Center, Penn State University, Biglerville, PA, U.S.A.
Phytopathology 100:S68

Bacterial plant pathogens have a major impact on crop production. *E. amylovora*, a gram-negative bacterium, is the causal agent of fire blight. Fire blight is the most significant and destructive bacterial disease of rosaceous plants, including economically important fruit crops such as apples and pears. This study quantifies disease severity caused by six *E. amylovora* isolates (Ea273, CFBP1367, Ea581a, E2002a, E4001a and Ea06P-1) on apple trees and seedlings. Isolates produced a range of disease severity, with Ea06P-1 producing the greatest disease severity in every assay. We determined the variation in virulence characteristic expression, including growth rates in immature apple fruit, amylovoran production, levansucrase activity, biofilm formation, carbohydrate utilization, hypersensitive cell death elicitation, and protein secretion profiles. Multiple regression analysis indicated that amylovoran production, biofilm formation and growth in immature apple fruit accounted for over 70% of the variation in disease severity on apple seedlings. Furthermore, in greenhouse-grown 'Gala' trees, over 75% of the variation in disease severity was accounted for by amylovoran production, biofilm formation, growth in immature apple fruit, hypersensitive cell death elicitation, and sorbitol utilization. This study demonstrates that virulence factor expression levels account for differences in disease severity caused by wild isolates of *E. amylovora* on apple trees.

Functional identification of *Xylella fastidiosa* plasmid replication and stability factors
M. LEE (1), E. E. Rogers (1), D. C. Stenger (1)
(1) USDA ARS, Parlier, CA, U.S.A.
Phytopathology 100:S68

Xylella fastidiosa (Xf) strain RIV11 harbors a 25 kbp plasmid (pXFRIV11) belonging to the incP1 incompatibility group. Replication and stability factors of pXFRIV11 were identified and used to construct plasmids able to propagate in both Xf and *Escherichia coli*. Sequences required for replication in *E. coli* and conferring antibiotic resistance were derived from the cloning vector pCR2.1. Replication in Xf required a 1.35 kbp region from pXFRIV11 containing a replication initiation gene (*trfA*) and the adjacent origin of DNA replication (*ori*V). This region also conferred plasmid replication in *Agrobacterium tumefaciens*, *Xanthomonas campestris*, and *Pseudomonas syringae*. Constructs containing the *trfA* gene and *ori*V derived from pVEIS01, a similar 31 kbp incP1 plasmid of the earthworm symbiont *Verminephropbacter eiseniae*, also were competent for replication in Xf. As expected, constructs bearing only *trfA* or *ori*V from either incP1 plasmid were unable to replicate in Xf. Although these incP1 replicons could be maintained in Xf under antibiotic selection, removal of selection resulted in loss of the plasmid. A novel toxin/antitoxin (*pem*I/*pem*K) addiction system of pXFRIV11 was added, improving stability of incP1 replicons in Xf in the absence of antibiotic selection. The resulting 6 kbp Xf shuttle vector (pXF20-PEMIK)

also contains 10 unique endonuclease recognition sites for insertion of foreign DNA.

Finer differentiation of phytoplasma strains based on phylogenetic analysis of the *secY* gene

I. LEE (1), K. D. Bottner-Parker (1), Y. Zhao (1), R. E. Davis (1), N. A. Harrison (2)
(1) USDA ARS MPPL, Beltsville, MD, U.S.A.; (2) FLREC, University of Florida, Ft. Lauderdale, FL, U.S.A.
Phytopathology 100:S69

The 16S rRNA gene has been widely used as a molecular marker for differentiation of phytoplasmas. Earlier studies indicated that 16S rRNA gene-based phylogenetic analysis was sufficient to classify phytoplasmas into 16Sr groups and subgroups, but its efficacy for differentiation of closely related strains within a subgroup was relatively limited. Additional markers are needed to overcome this limitation. The protein translocase gene, *secY*, is more variable than the 16S rRNA gene and may represent a potential marker. Comparative phylogenetic analyses with 16S rRNA and *secY* gene sequences from representative phytoplasma strains were performed to assess the efficacy for delineating phytoplasma strains within each 16Sr group and subgroup. The phylogenetic interrelatedness among phytoplasma taxa inferred by *secY* gene-based phylogeny was nearly congruent with that inferred by 16S rRNA gene-based phylogeny. However, the *secY* gene-based phylogeny not only readily resolved 16Sr subgroups within a given 16Sr group, but also delineated distinct lineages irresolvable by 16S rRNA gene-based phylogeny. Such high resolving power makes the *secY* gene a more useful genetic marker than the 16S rRNA gene for finer differentiation of closely related phytoplasma strains based on collective RFLP patterns using select restriction enzymes. The genetic interrelationships among these strains thus determined coincided with delineations by phylogenetic analysis.

Characterization of four Mitogen-activated protein kinase genes (*CsMPS1*, *CsHOG1*, *CsCHK1* and *CsSte11*) in the fungal cereal pathogen, *Cochliobolus sativus*

Y. LENG (1), S. Zhong (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S69

Mitogen-activated protein kinase (MAPK) pathways have been demonstrated to be involved in fungal development, sexual reproduction and/or virulence in several filamentous plant pathogenic fungi, including *Cochliobolus heterostrophus* and *Magnaporthe grisea*, but genes for MAPKs in *Cochliobolus sativus*, the causal agent of spot blotch, common root rot and black point in barley and wheat, have not been characterized. We identified four MAPK genes (*CsMPS1*, *CsHOG1*, *CsCHK1* and *CsSte11*) in the whole genome sequence of the *C. sativus* isolate ND93-1 based on their homology to MAPK genes characterized in other fungi. Gene knockout mutants were generated for *CsSte11* and *CsCHK1* and are being generated for the other two genes (*CsMPS1* and *CsHOG1*) using the split marker system. The knockout mutants of *CsSte11* and *CsCHK1* were defected in conidiation, indicating that they are involved in the asexual development of *C. sativus*, as *ChSte11* and *CHK1* of *C. heterostrophus* do, respectively. However, *CsCHK1* appears not to be involved in melanization because no difference was observed between the *CsCHK1* mutant and the wild type in colony pigmentation on PDA plates. Thus, *CsCHK1* is different from the *CHK1* of *C. heterostrophus* in that the latter is involved in regulation of the melanin synthesis. The results of pathogenicity tests and other phenotypic changes of the knockout mutants in comparison with the wild type isolate will be presented.

A multiplex TaqMan assay for detection and differentiation of *Leptosphaeria maculans* and *L. biglobosa*, causal agents of canola blackleg

W. Leonard (1), X. LI (1), D. S. Smith (1), J. Nie (1), T. Dumonceaux (2)
(1) Canadian Food Inspection Agency, Charlottetown, PE, CANADA; (2) Agriculture and Agri-Food Canada, Saskatoon, SK, CANADA
Phytopathology 100:S69

Blackleg of canola is caused by two related fungal species, *Leptosphaeria maculans*, a highly virulent species causing severe cankers at the base of stems, and *L. biglobosa*, a weakly virulent species causing mild stem lesions. For rapid detection and differentiation in canola seed, we designed a multiplex TaqMan assay that targeted two genomic loci of *L. maculans*, *LopB*, a pathogenicity gene and *LMR1*, a repetitive element linked to a virulence region, and the mating protein gene, *Mat1-2*, of *L. biglobosa*. The primers specifically amplified 110, 145, and 135 bp products from the three loci, respectively, and TaqMan probes were labelled with Quasar 670, Cal-Fluor-Red 610, or Cal-Fluor-Orange 560 fluorescent dyes and compatible quenchers, respectively. To validate negative results in the mutiplex PCR, a FAM-labelled probe to a 200-bp internal control, designed from a rice sequence bordered by *LopB* primer sequences, was included in the assay. The final assay involved a 48-hr enrichment of 4-g samples in a minimal medium with antibiotics, sample treatment in a beadbeater, and DNA extraction with magnesil paramagnetic beads prior to assay by multiplex PCR. Under optimized conditions the assay detected single infected seeds in a sample and differentiated between *L. maculans* and *L. biglobosa*. Positive detection and diagnosis was based on cycle threshold cutoff values and confirmation of amplicon identity by capillary electrophoresis analyses.

An Indiana survey of *Phytophthora* species in nurseries, greenhouses, and landscape plantings

A. J. LEONBERGER (1), C. Speers (1), G. Ruhl (1), T. Creswell (1), J. Beckerman (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S69

From 2006 to 2008, samples with symptoms consistent with Phytophthora blight and crown rot were collected as part of the USDA-APHIS *Phytophthora ramorum* nursery survey and submitted by additional outside sources to the Purdue Plant and Pest Diagnostic Laboratory. From 30 sites, 121 *Phytophthora* isolates were obtained from 1657 host samples containing 32 genera. Comparison of the internal transcribed spacer (ITS) sequence of the ribosomal DNA identified 9 *Phytophthora* sp. A majority of the isolates were either *P. citricola* (44.4%) or *P. citrophthora* (27.2%). *P. citricola* isolates were collected at 10 sites from 5 host genera, and included *Forsythia*, *Juglans*, *Pieris*, *Rhododendron*, and *Syringa*. *P. citrophthora* was isolated from 8 sites on 6 host genera: *Abies*, *Forsythia*, *Ilex*, *Pieris*, *Rhododendron*, and *Syringa.* The other identified *Phytophthora* sp. consisted of *P. cactorum*, *P. capsici*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae*, *P. palmivora*, *and P. syringae*. Sixteen isolates showed signs of possible species hybridization. Four isolates were found to be hybrids of *P. cactorum* and *P. hedraiandra* as verified by cloning and sequencing the ITS sequences. Three of the *P. cactorum x hedraiandra* 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. hedraiandra*, and suggests an increase of host range due to species hybridization.

Reduced sensitivity and complete resistance of Michigan *Venturia inaequalis* populations to sterol- and quinone-outside inhibitor fungicides

K. E. LESNIAK (1), T. J. Proffer (1), A. Irish-Brown (1), G. W. Sundin (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S69

Control strategies for *Venturia inaequalis* rely heavily on chemical applications. Single-site fungicides such as the quinone-outside inhibitors (QoI) and the sterol-inhibitors (SI) have been used in Michigan apple orchards for 11 and more than 20 years, respectively. In 2008, we detected the G143A mutation in the cytochrome *b* gene, conferring complete resistance to QoI's, in *V. inaequalis* isolates from 12 orchards located in two apple producing regions in MI. In 2009, >1,500 single monoconidial isolates were established from 66 orchards throughout Michigan and five non-orchard sites from Michigan and Ohio. These 2009 isolates were subjected to two assays; molecular detection of the G143A mutation using PCR, and a relative growth (RG) assay on SI-amended media using discriminatory dosages for SI sensitivity. To date, the G143A mutation has been detected in 63% of isolates from 59 Michigan orchards. Previous studies indicate that practical resistance to SI's occurs in an orchard when the mean RG of all isolates is >67%. In 50 of 54 orchards currently tested, the average RG was above that level. The mean RG's of isolates from three non-SI treated sites was 43%. These combined results indicate the immediate need for altered management strategies of *V. inaequalis* in Michigan orchards.

Biological and molecular characterization of a cucumber isolate of *Melon necrotic spot virus* from Ohio

D. LEWANDOWSKI (1)
(1) The Ohio State University, Columbus, OH, U.S.A.
Phytopathology 100:S69

An isolate of *Melon necrotic spot virus* (MNSV), designated MNSV-cucumber was collected from symptomatic cucumbers (*Cucumis sativus*) within a commercial greenhouse in Ohio in 2008. MNSV-cucumber was mechanically transmitted to *C. sativus*, *Cucumis melo*, and *Citrullus lanatus*, producing both local and systemic symptoms on some cultivars. MNSV-cucumber also produced local lesions on mechanically inoculated cotyledons of *Cucurbita moschata* and *Cucurbita pepo*. MNSV-cucumber was transmitted by an isolate of *Olpidium bornovanus* collected from the MNSV-infected cucumbers to cucumber, melon, and watermelon. The ITS region of the Ohio cucumber isolate of *O. bornovanus* was identical to a cucumber isolate collected in Spain. Total RNA extracted from MNSV-infected cucumber leaf tissue using the RNeasy Plant Mini Kit (Qiagen, Inc.) was amplified by RT-PCR using MNSV-specific primers. The genome of MNSV-cucumber was most similar to MNSV-Al, a melon isolate from Spain. The

MNSV-cucumber genome was 4,267 nts in length and had a typical MNSV genome organization, encoding five proteins: p29, p88, p7A, p7B, and p42 (coat protein). To the best or our knowledge, this is the first MNSV isolate from the United States to be sequenced and characterized, and only the second isolate from cucumber to be characterized.

Dual use research in the life sciences

P. LEWIS (1)
(1) Natl Inst of Health, Bethesda, MD, U.S.A.
Phytopathology 100:S70

Life sciences research is vital to improving public health, agriculture and the environment, while strengthening our national security and economy. However, the very same research may also yield information or technologies that could be misused for harmful purposes. For instance, information from certain life sciences research can be misapplied to create dangerous pathogens to threaten humans, animals, and plants. The development of new technologies and the generation of information with the potential for both benevolent and malevolent purposes is referred to as "dual use research." Scientists have a professional responsibility to be aware of dual use research issues and the potential ways in which information and products of their research could be misused, and to take steps to minimize misuse of their work. The National Science Advisory Board for Biosecurity (NSABB) was established by the U.S. Government to advise on strategies for dealing with potential dual use research. The NSABB has produced a series of reports on strategies for overseeing and educating about dual use research. In the future, the Board will continue to address topics of relevance to the plant pathology community, including: (1) enhancing the culture of responsibility that already exists among life sciences researchers and (2) advising on the HHS and USDA Select Agent Program, as requested.

Disruption of the salicylate and jasmonate signaling pathways by the cucumber mosaic virus 2b RNA silencing suppressor

M. G. Lewsey (1), A. M. Murphy (1), D. MacLean (2), N. Dalchau (3), J. Westwood (1), K. Macaulay (1), M. H. Bennett (4), M. Moulin (1), D. E. Hanke (1), G. Powell (4), A. G. Smith (1), H. Ziebell (5), J. CARR (1)
(1) University of Cambridge, Cambridge, UNITED KINGDOM; (2) Sainsbury Laboratory, Norwich, UNITED KINGDOM; (3) Microsoft Research, Cambridge, UNITED KINGDOM; (4) Imperial College, London, UNITED KINGDOM; (5) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S70

The cucumber mosaic virus (CMV) 2b counter-defense protein disrupts plant antiviral mechanisms mediated by RNA silencing and salicylic acid (SA). We used microarrays to investigate defensive gene expression in *2b*-transgenic *Arabidopsis thaliana* plants. Surprisingly, 2b inhibited expression of few SA-regulated genes and in some instances enhanced the effect of SA on certain genes. Strikingly, the 2b protein inhibited changes in the expression of 90% of genes regulated by jasmonic acid (JA). Consistent with this, infection of plants with CMV, but not the *2b* gene deletion mutant CMVΔ2b, strongly inhibited JA inducible gene expression. JA levels were unaffected by infection with either CMV or CMVΔ2b. Although the CMV-*Arabidopsis* interaction is a compatible one, SA accumulation, usually considered to be an indicator of plant resistance, was increased in CMV-infected plants but not in CMVΔ2b-infected plants. Thus, the 2b protein inhibits JA signaling at a step downstream of JA biosynthesis but it primes induction of SA biosynthesis by another CMV gene product or by the process of infection itself. JA is important in plant defense against insects and CMV, like many plant viruses, is aphid-transmitted. This raises the possibility that disruption of JA-mediated gene expression by the 2b protein may influence CMV transmission. Funded by grants from the BBSRC and Leverhulme Trust.

Detection and identification of pectobacteria associated with potato blackleg reveals the presence of mixed populations

X. LI (1), L. Ward (1), J. Nie (1), J. Nickerson (1), A. MacDonald (1), J. Gourley (1), S. H. De Boer (1)
(1) Canadian Food Inspection Agency, Charlottetown, PE, CANADA
Phytopathology 100:S70

The causal agents of the potato stem rots, blackleg and aerial stem rot, were recently reclassified on their phenotypic and phylogenetic characteristics as *Pectobacterium atrosepticum* (PA) and *P. carotovorum* (PC). Moreover, *P. brasiliensis* (PB) and *Dickeya* spp. (DI) are now also recognized as causal agents of blackleg-like symptoms in potato in Brazil and Europe, respectively. In a preliminary survey of pectobacteria associated with blackleg symptoms on potato in Canada, not only were PA and PC detected using PCR with taxon specific primers and direct isolation, but *P. wasabiae* (PW), originally described as the causal agent of soft rot of horse radish, was also detected. Our study revealed that the most common pectobacteria associated with potato blackleg in Canada was PA (85% of the total stem samples with blackleg symptom). However, PW was also frequently present (36%, often in the same stems as PA), while PB (3%) and DI (2%) were found occasionally. To detect and identify pectobacteria directly in diseased stems, strategies were developed including a multiplex PCR assay targeting various genomic regions of PA, PB, PC, PW and DI, and a rapid LAMP protocol for detecting and identifying PA. The LAMP protocol targets the genomic region encoding the pathogenicity-related polyketide synthase gene. The assay was specific for all strains of the species tested and showed no cross-amplification of related pectobacteria, dickeya strains, or other selected plant pathogenic bacteria.

Isolation and application of a *Bacillus subtilis* strain to control gray mold on tomato and powdery mildew on cucumber

S. LI (1), X. Lu (1), B. Li (1), Q. Guo (1), Z. Li (1), P. Ma (1)
(1) Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, Baoding, Hebei, PRC PEOPLES REP OF CHINA
Phytopathology 100:S70

Gray mold and powdery mildew are important diseases on tomato and cucumber respectively causing a great damage in China. The control of both diseases 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 humans, wildlife, foods, pathogen resistance and environment. So, it is a hot research point nowadays to find and use natural product to control these diseases. Microbial fungicides show such a more potential efficacy of control on plant diseases in greenhouse that the studies thereof become more popular in China. In this study, a bacterial isolate *Bacillus subtilis* BAB-1 were screened against the pathogen *Botrytis cinerea* by dual culture and showed a significant efficacy to control gray mold on tomato in pot test. A preparation, 5×10^8 cfu/ml spores AS, was made with spores of *B. subtilis* BAB-1 and applied in greenhouse. Naturally infested, the tomato and cucumber at flowering stage were sprayed 4 times (interval 7 days) with 50-fold diluted solution of this preparation. 45 days after first spraying, the diseases were rated. This experiment was designed randomly and conducted in two years, 4 replicates for each year. The treatment could significantly reduced both diseases at $p = 0.05$ with a control efficacy 81.6%–93.7% on gray mold of tomato and 81.1%–98.3% on powdery mildew of cucumber. This study will provide a new and environment-friendly fungicide to control both diseases in China.

Screening germplasm for resistance to Phomopsis seed decay in soybean

S. LI (1), J. C. Rupe (2), P. Chen (2), A. Wrather (3)
(1) USDA ARS CGRU, Stoneville, MS, U.S.A.; (2) University of Arkansas, Fayetteville, AR, U.S.A.; (3) University of Missouri, Portageville, MO, U.S.A.
Phytopathology 100:S70

Phomopsis longicolla is the primarily cause of soybean Phomopsis seed decay (PSD), a major cause of poor seed quality in the United States. To identify new sources of soybean lines resistant to PSD, field screening of 135 selected soybean germplasm lines representing 28 worldwide origins and maturity groups 3-5 along with PSD resistant and susceptible checks were tested in Arkansas, Missouri, and Mississippi. Each entry was grown in a single 3-m row plot in a randomized complete block design with four replications. Frequent rainfall during seed maturation led to high levels of seed infection by a number of fungi. Significant differences in seed infection by *P. longicolla* occurred among soybean lines with some lines having no PSD while others had levels as high as 90%. These differences between lines were reflected in visual seed quality and in seed germination. Several lines with low disease incidence, good visual quality, and high germination rate at all locations will be tested for resistance in 2010 field trials.

Insights into common functional domains of tospovirus NSm proteins

W. Li (1), D. J. Lewandowski (2), M. E. Hilf (3), S. ADKINS (3)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) The Ohio State University, Columbus, OH, U.S.A.; (3) USDA ARS USHRL, Ft. Pierce, FL, U.S.A.
Phytopathology 100:S70

Direct demonstration of tospovirus gene function has been impeded by the absence of reliable reverse genetics systems for this virus genus. Use of a *Tobacco mosaic virus* (TMV)-based expression system has demonstrated that the *Tomato spotted wilt virus* (TSWV) NSm protein supports cell-to-cell movement in the absence of other TSWV proteins. Essential TSWV NSm domains required for tubule formation, movement and symptoms were identified previously by deletion-mapping and alanine-substitution mutagenesis using the TMV-based system. Our mutagenesis studies of TSWV NSm amino acids that are conserved in other tospovirus NSm proteins suggest that functional domains may be conserved across the genus, especially for species in the 'New World' group. To that end, we initiated studies of the *Impatiens necrotic spot virus* (INSV) NSm protein using similar TMV-based mutagenesis approaches and we report here new insights into the tospovirus NSm protein based on our functional analysis of the INSV NSm protein.

Evaluation of resistance to *Pucciniastrum hydrangeae* in *Hydrangea arborescens*

Y. LI (1), M. Windham (2), R. Trigiano (2), A. Windham (3), S. Reed (4), T. Rinehart (5), J. Spiers (5)
(1) Connecticut Agric Experiment Station, New Haven, CT, U.S.A.; (2) University of Tennessee, Knoxville, TN, U.S.A.; (3) University of Tennessee, Nashville, TN, U.S.A.; (4) USDA/ARS, McMinnville, TN, U.S.A.; (5) USDA/ARS, Poplarville, MS, U.S.A.
Phytopathology 100:S71

Smooth hydrangea, *Hydrangea arborescens* L., is a native shrub in the eastern North America. Rust caused by *Pucciniastrum hydrangeae* (B. & C.) Arth. is an important disease on smooth hydrangeas in nurseries, gardens and landscapes. In order to understand resistance to the rust disease in smooth hydrangea, pustule formation and sporulation of the fungus on *H. arborescens* 'Annabelle', 'Frosty', 'Green Dragon', 'Mayes Starburst', 'Pink Pincushion', 'Ryan Gainey', and 'White Dome' were evaluated using a leaf disk bioassay. Variation in resistance to rust in *P. arborescens* was observed although formation of uredia and urediniospores was present on all seven cultivars. The cultivar 'Frosty' displayed the highest resistance and had a relatively small amount of uredia and urediniospore production. 'Green Dragon' was highly susceptible and permitted the formation of the largest number of uredia per disk. Intermediate reactions were detected with the other five cultivars, in which 'Mayes Starbust' and 'Annabelle' had significantly larger numbers of uredia than 'Ryan Gainey', 'Pink Pincusion' and 'White Dome'. These results provide important information for breeders to develop smooth hydrangea cultivars with rust resistance and for landscape designers and nurserymen to select smooth hydrangea for gardens and nursery production.

Biofilm formation and virulence associated with lipopolysaccharide biosynthetic genes in *Xanthomonas axonopodis* pv. *citri*

J. LI (1)
(1) Citrus Research and Education Center, University of Florida IFAS, Lake Alfred, FL, U.S.A.
Phytopathology 100:S71

Xanthomonas axonopodis pv. *citri* (Xac) is the bacterial pathogen of citrus canker which is one of the most disastrous diseases of citrus worldwide. The formation of biofilms in planta plays an important role in the epiphytic survival of Xac prior to development of canker disease. Lipopolysaccharide (LPS) has been indicated to be involved in biofilm formation and an important virulence factor in various animal and plant-associated bacteria. LPS is also being increasingly recognized as a major pathogen associated molecular pattern (PAMP) for plants inducing plant defense responses. Very little is known about the relationship between LPS and biofilm formation and virulence of Xac. In this study, six Tn5 insertion mutants of Xac strain306 with disrupted putative LPS biosynthetic genes of were examined. The LPS profiles of the six mutants were determined by SDS-PAGE analysis. The ability to form biofilm on plastic and glass surface was impaired in all the six mutants. In planta tests, the grapefruit seedlings inoculated with LPS-defect mutants by spraying showed reduced symptoms on leaves compared with those treated with the wild type strain306. The affected phenotypes tested could be restored fully or partly to those of the wild type by introducing the intact genes into the respective mutants. These findings suggest that LPS production is vital to biofilm formation and virulence of Xac.

***Colletotrichum acutatum* forms a novel internal infection cushion-like structure in the cuticle layer of chili pepper**

C. LIAO (1), K. Kuo (2), J. Wang (3), M. Lee (1)
(1) Department of Plant Pathology, National Chung Hsing University, Taichung, TAIWAN; (2) Department of Plant Protection, Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan, Taipei, TAIWAN; (3) The World Vegetable Center, P.O. Box 42, Shanhua, Tainan, TAIWAN
Phytopathology 100:S71

Fruit anthracnose of *Capsicum* spp. caused by *Colletotrichum acutatum* is a severe disease in chili pepper in Taiwan. To understand how the pathogen infects the plant, the infection process of *C. acutatum* on resistant and susceptible fruits were studied using three isolates with various virulences on chili pepper. Surface attachment, germination, appressorium formation and turgor pressure accumulation of appressorium were analyzed *in vitro*. The isolate with the lowest virulence showed less attachment ability and turgor pressure accumulation compared to the other two isolates. However, the three isolates formed penetration hyphae in the epidermal cells of susceptible hosts at 72 hours post-inoculation (hpi). Interestingly, this fungus forms a novel internal infection cushion-like structure on resistant and susceptible chili pepper fruits before penetrating the epidermal cell. The internal infection structure was located in the cuticle layer and was demonstrated by light and flurorecent microscopy with GFP-tagged transformants. The internal infection cushion-like structure formed within 24 hpi and kept growing in the cuticle layer and subsequently penetrated into the epidermal cell at 72 hpi. Formation of this internal structure by *C. acutatum* appears to be a response to the thick cuticle layer of pepper fruits. This fungus also formed similar internal structures on resistant hosts. However, the structure turned dark at 13 days post-inoculation and no further infection was observed.

A novel bipartite launch system for a potexviral vector suitable for either protein expression or virus-induced gene silencing (VIGS)

H. Lim (1), A. Vaira (2), L. L. Domier (3), J. HAMMOND (1)
(1) USDA ARS MPPL, Beltsville, MD, U.S.A.; (2) CNR, IVV, Torino, ITALY; (3) USDA ARS, Urbana, IL, U.S.A.
Phytopathology 100:S71

We have developed plant virus-based vectors for virus-induced gene silencing (VIGS) and protein expression, based on *Alternanthera mosaic virus* (AltMV), for infection of a wide range of host plants including *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Glycine max* (soybean). Infection may be established by either mechanical inoculation of *in vitro* transcripts or *via* agroinfiltration. *In vivo* transcripts produced by co-agroinfiltration of bacteriophage T7 RNA polymerase resulted in T7-driven AltMV infection from a binary vector in the absence of the *Cauliflower mosaic virus* 35S promoter. An artificial bipartite viral vector delivery system was created by separating the AltMV RNA-dependent RNA polymerase and Triple Gene Block (TGB)123-Coat protein (CP) coding regions into two constructs each bearing the AltMV 5′ and 3′ non-coding regions, which recombined *in planta* to generate a full-length AltMV genome. Substitution of TGB1 L(88)P, and equivalent changes in other potexvirus TGB1 proteins, affected RNA silencing suppression efficacy and suitability of the vectors from protein expression to VIGS.

A single amino acid substitution in PthA of *Xanthomonas axonopodis* pv. *citri* altering canker formation on grapefruit leaves

H. LIN (1), S. Hsu (2), K. Tzeng (2)
(1) Chungchou Institute of Technology, Changhua County, TAIWAN; (2) National Chung-Hsing University, Taichung, TAIWAN
Phytopathology 100:S71

Three novel atypical symptom-producing variants of *Xanthomonas axonopodis* pv. *citri* (*Xac*) were described recently in Taiwan. Only the variant designated as A^f type produces typical erumpent canker lesions on Mexican lime (*Citrus aurantifolia*) but induces flat necrotic with water-soaked margin lesions on grapefruit leaves (*C. paradisi*). Two homologous *pthA* were cloned and characterized from strains XW19 (a typical canker lesion producing strain) and XW47 (a strain of A^f type). The *pthA* homolog from XW19 was transformed into XW47. The transformant induced typical erumpent canker lesions on grapefruit leaves. Sequence analyses revealed over 99% homology in nucleotide and deduced amino acid sequences compared with *pthA* homologs deposited in GenBank. The amino acid residues located at positions 49, 286, 742 and 767 of PthA were different between XW47 and XW19. The PthA mutants with a single amino acid substitution at each of these four positions were constructed by site-directed mutagenesis. Modified PthA (S286P) from XW47 in transformant 47SP induced erumpent canker lesions on grapefruit leaves, whereas another modified PthA (P286S) from XW19 in transformant 47PS only induced flat necrotic lesions. These results suggested that a single amino acid substitution from either serine to proline or proline to serine at position 286 of PthA can alter canker formation by *Xac* on grapefruit leaves.

Development of a multiplex real-time RT-PCR assay for simultaneous detection of three pome fruit viroids

L. LIN (1), R. Li (1), R. Mock (1), G. Kinard (1)
(1) USDA ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S71

Several viroids infect pome fruit trees and their control is based primarily on quarantine and certification programs to distribute clean stock material. A multiplex, single tube TaqMan RT-PCR assay was developed to simultaneously detect *Apple scar skin* (ASSVd), *Pear blister canker* (PBCVd), and *Apple fruit crinkle* (AFCVd) viroids. Total nucleic acid extracts were prepared from healthy and infected pome fruit trees using a CTAB method. For each of the three viroids, a pair of primers and a probe labeled with a specific fluorescent reporter dye were designed and evaluated in both simplex and multiplex Taqman RT-PCR assays. The optimum primer combinations and concentrations were determined for the multiplex TaqMan RT-PCR assay using extracts, including mixed extracts, from trees infected with one of the viroids. The extract dilution end points for detecting each viroid were 10^{-5}, 10^{-4}, and 10^{-2} for AFCVd, ASSVd, and PBCVd, respectively, in the multiplex format. The method was further validated using samples from trees inoculated with all of these viroids. The results indicate multiplex TaqMan RT-PCR is capable of specifically detecting the presence of and differentiating each viroid in pome fruit trees with mixed viroid

infections. This assay could be very useful as a fast and sensitive complement to existing diagnostic methods for pome fruit viroids. This technique is particularly applicable to quarantine and certification programs where many samples need to be tested.

One-step multiplex RT-PCR for simultaneous detection of four viroids affecting pome fruit trees

L. LIN (1), R. Li (1), R. Mock (1), G. Kinard (1)
(1) USDA ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S72

Apple scar skin (ASSVd), *Apple dimple fruit* (ADFVd), *Apple fruit crinkle* (AFCVd), and *Pear blister canker* (PBCVd) viroids naturally infect pome fruit trees. These viroids are distributed worldwide and are important quarantine pathogens for the international movement of germplasm. A single-step multiplex reverse transcription polymerase chain reaction assay (mRT-PCR) was developed for the simultaneous detection of these viroids. Total nucleic acids were prepared from fruit trees infected with individual viroids and used as templates for mRT-PCR for either single or mixed viroid detections. Four pairs of viroid-specific primers were designed to amplify products of different sizes that were discernible by gel electrophoresis. The expected products of 371 bp for AFCVd, 270 bp for ADFVd, 186 bp for ASSVd and 120 bp for PBCVd were obtained in the both simplex and multiplex RT-PCRs. The sensitivities, specificities and efficiencies of mRT-PCR for detecting all four viroids were very similar to the individual simplex RT-PCR. The method was validated using samples from pome trees inoculated with all four of viroids. All viroids could be detected from infected pear trees and up to two viroids could be detected from apples. These techniques proved to be a simple, rapid and cost-effective means to detect these viroids in fruit trees. The procedure is especially applicable to certification and quarantine programs, where numerous samples need to be tested for all four viroids.

Extracellular *Xylella fastidiosa* genomic DNA enhances biofilm formation *in vitro*

H. LIN (1), D. M. Cheng (1), E. L. Civerolo (1)
(1) USDA-ARS PWA, Parlier, CA, U.S.A.
Phytopathology 100:S72

Xylella fastidiosa (*Xf*) is a Gram negative, xylem-limited bacterium that causes Pierce's Disease (PD) of grapevine, as well as other diseases of economically important crops and landscape plants. Many bacteria produce large amounts of extracellular DNA, which may function as a matrix component in biofilms. Biofilm formation is essential for *Xf* establishment *in planta*. However, factors affecting *Xf* biofilm biogenesis *in planta* are not completely understood. The objective of this study was to determine if extracellular genomic DNA is involved in the *Xf* biofilm formation *in vitro*. The relative amounts of extracellular DNA were positively correlated with planktonic growth and biofilm formation *in vitro*, but were negatively correlated with cell viability. DNase I treatment of actively growing *Xf* cultures in culture medium decreased or inhibited biofilm formation. In contrast, addition of *Xf* genomic DNA promoted biofilm formation. These results suggest that biogenesis of extracellular DNA may play a role for *Xf* biofilm formation and could be a critical step in establishment of host-bacterium interaction.

A new molecular diagnostic tool for quantitatively detecting and genotyping "*Candidatus* Liberibacter species"

H. LIN (1), H. Liao (2), Y. Bai (2), E. L. Civerolo (1)
(1) USDA ARS PWA, Parlier, CA, U.S.A.; (2) Guangxi Academy of Agricultural Sciences, Nanning, PRC PEOPLES REP OF CHINA
Phytopathology 100:S72

A new molecular diagnostic method was developed for quantitative detection of "*Candidatus* Liberibacter" species associated with citrus Huanglongbing ("*Ca*. Liberibacter asiaticus", "*Ca*. Liberibacter africanus" and "*Ca*. Liberibacter americanus") and potato zebra chip disorder ("*Ca*. Liberibacter solanaceraum"). This detection system employs a pair of universal primers designed in sequences conserved among the four Liberibacter species. Polymorphism due to deletions, insertions and nucleotide substitutions in the amplicons among the four Liberibacter species can be distinguished based on high resolution melting curve analyses. In contrast to multiplex PCR or multiple sets of primers or primers/probe commonly used for detection of different Liberibacter species, this diagnostic system, using only one pair of primers, greatly simplifies detection and eliminates competition between primer/primer and / or primer/probe combinations that may occur in multiplex systems. The assay is robust and cost-effective for reliable detection, quantification and identification of Liberibacter species in plants and insect vectors. This new diagnostic system is suitable for high throughput screening for regulatory and epidemiological studies in locations where multiple Liberibacter species may be present.

Bioinformatic analysis of genome sequence data for Ca. Liberibacter asiaticus

M. LINDEBERG (1), S. Saha (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S72

Ca. Liberibacter asiaticus (Las) is a bacterium vectored by psyllid insects and believed to be the causal agent of citrus greening. However, given the difficulty in culturing this organism, much about its biology remains a mystery. Availability of genome sequence data for a single isolate of Las (Duan et al, 2009), provides the opportunity to use sequence analysis to gain insights into its mechanisms of adaptation to host and vector. Approaches include characterization of metabolic pathways and comparison with those of bacteria either phylogenetically related or inhabiting analogous environmental niches. Mapping of extragenic elements such as promoters can additionally pinpoint genes for which coordinate regulation mediates adaptation to the host environment. Regulatory proteins of particular interest include RpoH, associated with response to elevated temperature and RirA, mediating cell uptake of iron. Knowledge of regulatory binding sites and other repetitive sequences is also useful when designed primers for strain detection. Results of genomic analysis of Ca. Liberibacter asiaticus, guides for genome viewing, and links to various online resources and genome analysis tools can be found at the Citrus Greening-HLB Genome Resources Website (http://citrusgreening.org)

Development and application of a single-tube immunocapture real-time PCR technology for sensitive detection of a panel of viruses in crop plants

K. LING (1), C. Feng (2), J. Q. Xia (3)
(1) USDA ARS, Charleston, SC, U.S.A.; (2) University of Arkansas, Fayetteville, AR, U.S.A.; (3) AC Diagnostics, Inc., Fayetteville, AR, U.S.A.
Phytopathology 100:S72

Enzyme-linked immunosorbent assay (ELISA) is the most widely used technology for plant virus detection. Its sensitivity however may not be satisfactory in detecting viruses in tissues with early infection, seeds or woody plants. Recently, real-time PCR has been introduced for plant virus detection with higher sensitivity. Immunocapture (IC) real-time PCR is a combination of these two technologies which traps virus particles by immunocapture and washes away PCR inhibitors, followed by real-time PCR in the same tube for virus detection. The objectives of this study are to evaluate the factors affecting the immunocapture capabilities in PCR tubes and to develop sensitive real-time PCR to a panel of viruses. Sixteen PCR tube types were evaluated for their ability to immunocapture. Computer-assisted sequence analysis was used to select the most conserved genomic regions for primer and probe design. The target viruses captured on the PCR tubes were comparatively analyzed with ELISA, PCR and real-time PCR. PCR tubes for the most effective immunocapturing of target viruses were identified. The IC real-time PCR could effectively detect 14 target viruses of tomato or pepper in crude tissue extract diluted up to 10^{-5} or 10^{-7}, which is approximately 1000 times higher than ELISA. Furthermore, multiplex IC real-time RT-PCR was developed for a simultaneous detection of 2–3 viruses. This technology has a potential to supplement ELISA for plant virus detection.

Transmission of cucumber green mottle mosaic virus by cucumber pollen

H. LIU (1), L. Luo (1), J. Hao (2), J. Li (1)
(1) China Agricultural University, Beijing, PRC PEOPLES REP OF CHINA; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S72

It has been postulated that pollen may transmit cucumber green mottle mosaic virus (CGMMV) in cucumber. To confirm this hypothesis, laboratory and greenhouse experiments were conducted. Cucumber seeds free of CGMMV were grown in the greenhouse. Plants were covered with screens to exclude insects, and divided into two blocks, with 22 plants in block one, and 109 plants in block two. When cucumber plants in block one had three true leaves, they were mechanically inoculated with CGMMV. At blooming stage, leaves from inoculated plants were collected and tested for CGMMV using reverse transcription polymerase chain reaction (RT-PCR). All leave samples tested positive for CGMMV. Pollen grains from the inoculated plants in block one were collected and used to artificially pollinate 109 emasculated cucumber plants in block two. Fruit were harvested from 45 of these plants. One cucumber fruit was selected from each plant, and the seeds were removed. RT-PCR test indicated that 24 of the 45 seeds sets (54%) were CGMMV positive. The 24 sets of CGMMV-positive seeds were planted in 24 groups of pots. Six seedlings from each group were tested for CGMMV using RT-PCR. Seedlings from nineteen groups (80%) were CGMMV positive. Therefore, CGMMV can be transmitted from plant to plant via cucumber pollen and is seed-borne disease.

Comparison of fumigation, mustard meal (MM) amendments and grafting on Fusarium and Pythium communities in tomato fields

B. LIU (1), J. Sun (2), F. Louws (2)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) PLPA, NCSU, Raleigh, NC, U.S.A.
Phytopathology 100:S73

Community analysis of soil *Pythium* and *Fusarium* is essential for understanding pathogen ecology and the impact of fumigation, MM and grafting on pathogen communities. The dynamics in microbial communities was monitored in the following treatments at the NC Mountain Horticulture Research Station: inactive-MM with no-grafting (a); methyl bromide:chloropicrin (MC) fumigation combined with grafting on Maxifort rootstock (b) or with no-grafting (c); MM with (d) or with no grafting (e); no-fumigation with (f) or with no grafting (g) and no-fumigation with self-grafting (h). Soil samples were assessed in July (before planting), September (full bloom) and October (full fruiting). Based on dilution plating, Fusarium populations were not significantly different in soils from the first collection (before treatment) and third sampling (Oct). At the second sampling (Sept), Fusarium populations were reduced significantly in soils with treatment d (62 CFU/gds) and increased in soils with treatment b (3.0 × 104 CFU/gds) and c (4.6 × 104 CFU/gds) compared with no fumigation baseline f, g and h (7 × 102 to 2.5 × 103 CFU/gds). Moreover, at the second (Sept) and third sampling (Oct), Pythium populations were decreased significantly in treatment b (21 and 43 CFU/gds) and c (16 and 12 CFU/gds) but not by other treatments compared with baseline level f, g, and h [1.5 × 102 to 3.1 × 102 (Sept) and 6.2 × 102 to 8.7 × 102 CFU/gds (Oct)]. The differential impact of MM and fumigation on Fusarium and Pythium populations is a productive avenue for future research.

Comparison of fumigation, mustard meal amendments and grafting on bulk soil microbial communities in tomato fields

B. LIU (1), J. Sun (2), C. Rivard (2), R. Welker (2), F. J. Louws (2)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) PLPA, NCSU, Raleigh, NC, U.S.A.
Phytopathology 100:S73

Plant health benefits associated with soil fumigation, mustard meal (MM) amendments and tomato grafting may be mediated by microbial communities. The dynamics and shifts in microbial communities was monitored in the following treatments at the NC Mountain Horticulture Research Station: inactive-MM with no-grafting (a); methyl bromide:chloropicrin (MC) fumigation combined with grafting on Maxifort rootstock (b) or with no-grafting (c); MM with (d) or with no grafting (e); no-fumigation with (f) or with no grafting (g) and no-fumigation with self-grafting (h). Soil samples were assessed in July (before planting), September (full bloom) and October (full fruiting). Based on dilution plating, fungal populations did not differ significantly in soils from the first collection (before soil treatment) and third sampling (Oct). At the second sampling (Sept), fungal populations were significantly increased in soils with treatment b (3.3 × 105 CFU/ gram dry soil) and c (3.9 × 105 CFU/gds) compared with no-fumigation baseline f, g and h (81 to 2.4 × 102 CFU/gds). Moreover, total bacterial populations were decreased in treatment b and d but not by other treatments compared to baseline levels in f, g, and h. Denaturing gradient gel electrophoresis is in progress to characterize fungal and bacteria species diversity in soils with different treatments. Knowledge about the biology and ecology of microbial communities could provide a more informed framework to advance plant health and disease management programs.

Variation of prophage frequency in "Canidatus Liberibacter asiaticus" strains from two geographical distinct citrus growing Provinces in China

R. Liu (1), P. Zhang (1), J. Chen (2), X. DENG (1)
(1) South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA; (2) USDA ARS PWA, Parlier, CA, U.S.A.
Phytopathology 100:S73

Prophages are important genetic elements of bacterial genomes and are involved in lateral gene transfer, pathogenicity, environmental adaptations and interstrain genetic variability. In this study, the sequence of a phage terminase gene of "Candidatus Liberibacter asiaticus", a bacterium associated with citrus Huanglongbing (HLB), was identified and used to represent the corresponding prophage. Based on the DNA sequence, a set of primers was designed and used for PCR detection of prophage in HLB citrus samples collected from two geographically distinct provinces, Guangdong with an average altitude of <500 m, and Yunnan with an average altitude of >2,000 m. The frequency of prophage detection was 15.8% (19/120) in Guangdong and 97.4% (38/39) in Yunnan. Chi-square analysis showed that the prophage frequencies between the two regions were significantly different ($P < 0.0001$). However, the prophage gene sequences obtained from 10 Guangdong strains and 8 Yunnan strains shared 100% similarity, suggesting identity or high similarity of the corresponding prophage. The absence of the terminase gene sequence in some "Ca. L. asiaticus" strains suggests that the prophage or putative phage has both lytic and lysogenic cycles. To our knowledge, this is the first observation on phage lytic activity in "Ca. L. asiaticus".

Molecular characterization of the mitochondrial cytochrome b gene in *Podosphaera clandestina*, the causal agent of powdery mildew on sweet cherry

Q. LIU (1), M. E. Nelson (1), G. G. Grove (1)
(1) Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S73

The mitochondrial cytochrome b gene (cytb) encodes a protein that is targeted by QoI (strobilurin) fungicides, which cause energy deficiency in fungal cells during spore germination. Mutations in the cytb gene, most commonly at positions 143 and 129, confer resistance to QoI fungicides in various phytopathogens including powdery mildews. The objective of current study was to develop a procedure for amplifying and characterizing the cytb gene of *Podosphaera clandestina*, the causal agent of cherry powdery mildew, so that putative mechanism for QoI fungicide resistance can be promptly determined once it arises. Using degenerate primers and nested polymerase chain reaction, products of expected size amplified from two greenhouse isolates were cloned and sequenced. The two amplicons were 641 bp and 638 bp in size, respectively. There were no introns within either amplicons. Among amino acids they encoded, 69–71% and 86–95% were identical to the corresponding regions of powdery mildew cytb genes deposited in GeneBank. Most variations between two amplicons occurred within those 100 amino acids at 3′ end. Amino acids at positions of 129 and 143 were phenylanlanine (F) and glycine (G), respectively, for both amplicons. These results provide sequence information for the *P. clandestina* cytb gene. This information will be useful to further investigation of QoI resistant *P. clandestina* isolates if that resistance is due to mutations in the cytb gene as has been reported in other fungi.

CANARY biosensors for rapid detection of *Ralstonia*, Potyvirus and *Phytophthora*

Z. LIU (1), K. Rappaport (1), E. Twieg (1), V. Mavrodieva (1), L. Levy (1)
(1) USDA-APHIS-PPQ-CPHST, National Plant Germplasm and Biotechnology Laboratory, Beltsville, MD, U.S.A.
Phytopathology 100:S73

CANARY (Cellular Analysis and Notification of Antigen Risk and Yield) is a cell-based technology that is capable of rapidly identifying low levels of pathogens through detection of photons emitted by bioluminescent proteins upon crosslinking of antigens to engineered antibodies expressed and anchored on the outer membrane of the cell. The method was invented by scientists at the Massachusetts Institute of Technology and has been applied for medical diagnostic assays and monitoring of select agents. MIT and USDA APHIS developed plant-pathogen-specific-cell lines for detection of *Ralstonia solanacearum*, and to the genus-level for Potyvirus and *Phytophthora*. The eventual goal is to implement the CANARY system in plant diagnostic labs. For *Ralstonia* detection, we sliced a small piece of infected plant tissue and soaked it in assay buffer for a few minutes before CANARY testing. As few as 3 CFUs of *Ralstonia* can be detected in a single test. For Potyvirus detection, we used polystyrene beads to capture viruses from plant extract for CANARY. For both the Potyvirus and *Ralstonia* assays, only 10 minutes or less are required for sample preparation and sample testing. For *Phytophthora* detection, a second antibody, which is different from cell membrane antibodies, was used to coat magnetic beads that were applied to capture mycelia in plant extract in preparation for CANARY. The protocols and data will be discussed to demonstrate the speed and sensitivity of this technology.

Involvement of rice endogenous peptide elicitors in defense signaling and disease resistance

W. LIU (1), Q. Wang (1), Y. Yang (1)
(1) Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S73

Plant disease resistance is generally activated by perception of pathogen-associated molecular patterns or effectors and frequently mediated by salicylic acid, jasmonic acid, ethylene and/or abscisic acid signaling pathways. However, relatively little is known about the role of endogenous plant peptide elicitors in defense signaling and disease resistance. Recently, a small family of *Arabidopsis* peptide elicitors (AtPeps) was shown to mediate defense gene activation and basal resistance. Here we have identified a 7-member family of rice peptide elicitor precursor genes (*OsPROPEP1-7*), which encodes a class of 25–42 amino-acid mature peptides. Based on publicly available rice microarray data and our quantitative RT-PCR analysis, at least three *OsPROPEPs* were significantly induced by fungal infection, wounding, jasmonic acid and/or ethylene treatments. A 25 amino-acid peptide (OsPep7)

was synthesized and is being tested for its activation of rice defense genes and resistance responses. In addition, transgenic rice lines with overexpression of OsPROPEP7 have been generated and will be characterized for altered defense gene expression and disease resistance against rice blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani*).

Identification of a conserved rice protein that interacts with a Nep1-like toxin from *Magnaporthe oryzae*

Z. LIU (1), Q. Wang (1), Y. Yang (1)
(1) Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S74

Necrosis and ethylene-inducing peptide1 (Nep1)-like proteins (NLPs) are conserved in a diverse array of microorganisms including bacteria, fungi and oomycetes and act as virulent toxins to elicit necrotic cell death in dicotyledonous plants. Recently, we have identified a family of four *Magnaporthe oryzae* NLPs (MoNLPs) that are capable of eliciting necrotic cell death in both dicots and monocots. To better understand the mode of action and potential host cellular target(s) of NLP toxins, MoNLP1 was used as a bait to screen for interacting rice proteins by the yeast two-hybrid system. Three interacting rice protein were identified, including OsNPI1 (Oryza sativa NLP Interactor1), which is highly conserved in eukaryotes. Quantitative RT-PCR revealed that OsNPI1 is constitutively expressed in rice plants during the infection of *M. oryzae*. Interestingly, knockout of the NPI1 orthologue in *Arabidopsis* T-DNA mutants led to retarded growth and lethal phenotype. In addition, suppression of OsNPI1 by RNA interference in transgenic rice appears to negatively affect shoot regeneration and plant growth. Functional characterization of OsNPI1 at the protein level and further analysis of suppression and overexpression transgenic lines should shed light on the role of OsNPI1 as a potential MoNLP1 target which mediates necrotic cell death and disease development.

Study on the influence on germination of resting spores of *Plasmodiophora brassicae* on pakchoi

Y. LIU (1), X. Huang (1), H. Liu (1)
(1) Sichuan Academy of Agricultural Sciences, Institute of Plant Protection, Chengdu, PRC PEOPLES REP OF CHINA
Phytopathology 100:S74

The germination of resting spores of *Plasmodiophora brassicae* on club root pathogen will cause different infection degree on crucifer. Understanding the restricting factor on germination of resting spores of *P. brassicae* become the key points to control the club root efficiently. The germination of resting spores of *P. brassicae* from pakchoi under different conditions were tested through liquid culture method, such as temperature, pH, nutrient condition, visible light and different rotted degrees of clubs. The result shown that the optimum temperature for germination of resting spores of *P. brassicae* was 24°C with suitable range from 20–28°C and the lethal temperature was 48°C; the optimum pH was 6.3 with suitable range from 6.0–6.7, the germination of resting spores was restrained by the visible light but motivated observably after the treatment of rotted clubs. The highest germination rate for the resting spores was 71.07% in the filtering sterilized root exudates, which shown that the germination of resting spores was motivated observably by tissue secretion.

Suppression of zinnia powdery mildew in the greenhouse with silicon-containing media amendments

J. C. LOCKE (1), J. E. Altland (2), J. M. Frantz (1)
(1) USDA-ARS-ATRU, Toledo, OH, U.S.A.; (2) USDA-ARS-ATRU, Wooster, OH, U.S.A.
Phytopathology 100:S74

We have previously reported that supplemental silicon supplied in hydroponic solution or soilless media can reduce the incidence and severity of powdery mildew (PM) on zinnia. This research reports the use of 1) silicon-containing organic amendments, 2) mineral compositions high in silicon content, and 3) drenched potassium silicate solution as delivery systems for silicon to zinnia grown in a peat-based growing medium. Efficacy varied with silicon concentration and/or availability in the amendments, amount of amendment supplied, and the disease pressure. Three of the amendments; rice (*Oryza sativa*) hulls (RH), chopped miscanthus grass (*Miscanthus x giganteum*) straw (MS), and chopped switchgrass (*Panicum virgatum*) straw (SG) reduced severity of PM ratings after three weeks but the degree of reduction decreased with increasing exposure time for all three. After 6 and 8 weeks, only RH and MS provided substantial reduction in disease. Declining protection correlates with a declining level of silicon in the zinnia leaf tissue and may correspond with a depleted silicon supply in the medium. Constant application of potassium silicate solution provided the best protection suggesting the need for a continuous supply of silicon to the leaf tissue and negating the potential of a slow-release supply of silicon from the amendments or an accumulated reserve of silicon in the zinnia tissue. Additional approaches are being explored to extend the usefulness of silicon supplementation in suppressing mildew.

Incidence of *Candidatus* Liberibacter americanus and *Ca.* L. asiaticus in orange jasmine and citrus trees in urban areas in Sao Paulo State, Brazil

S. LOPES (1), G. F. Frare (2), L. E. Camargo (2), N. A. Wulff (3), D. C. Teixeira (3), R. B. Bassanezi (3), A. C. Beattie (4), A. J. Ayres (3)
(1) FUNDECITRUS, Araraquara, BRAZIL; (2) Piracicaba, BRAZIL; (3) Araraquara, BRAZIL; (4) Sydney, AUSTRALIA
Phytopathology 100:S74

Liberibacters are phloem-limited bacteria associated with huanglongbing (HLB), a destructive citrus disease. Two species occur in Brazil, *Candidatus* Liberibacter asiaticus (Lam) and *Ca.* L. americanus (Las), both vectored by *Diaphorina citri*. Orange jasmine is a widespread ornamental tree and host of both liberibacters and *D. citri*. We determined liberibacter incidence in cities and villages within the most important Brazilian citrus belt, and their graft-transmissibility. To assess inoculum source potentials, quantitative PCR was applied to all PCR-positive trees. Liberibacter was detected in 91 of 786 trees distributed in 10 of 76 locations, all within the region of high HLB incidence. PCR-positive trees exhibited yellow shoots and/or dieback indistinguishable from those on PCR-negative trees. Among the PCR-positive trees, Lam was proportionally more frequent in the 2005/6 and Las in the 2009 survey. Transmission succeeded only in homologous host combinations, including Lam (2/10) from/to orange jasmine and Lam (5/18) and Las (5/9) from/to citrus. Symptoms were mild and progressed less in orange jasmine probably due to lower liberibacter multiplication. In this host, Lam and Las titers averaged 4.3 and 3.0 log cells/g tissue compared to 5.5 and 7.3 in citrus. Orange jasmine is not as suitable for liberibacter multiplication as citrus. However, its importance to the HLB epidemics should not be underestimated. It is a preferred host of *D. citri* and is not under any insect- or strict tree eradication-control program.

Distribution and diversity of *Pratylenchus* spp. associated with biofuel crops and species identification in a multiplex PCR assay

H. LOPEZ NICORA (1), T. Mekete (1), K. Reynolds (1), M. E. Gray (1), T. L. Niblack (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S74

The distribution of *Pratylenchus* spp. was surveyed in field plots of bioenergy crops in six states: Illinois, Iowa, South Dakota, Kentucky, Tennessee, and Georgia. The populations were identified based on morphology and morphometrics and further characterized based on sequences of the rDNA D2D3 region. The region revealed variations in sequence information that supported the morphological identification. In this work, 6 *Pratylenchus* spp. were detected: *P. scribneri*, *P. penetrans*, *P. crenatus*, *P. hexincisus*, *P. neglectus*, and *P. brachyurus*. *P. scribneri*, *P. crenatus*, and *P. penetrans* were distributed most widely with detection rates up to 34%, 29% and 15%, respectively. *P. hexincisus*, *P. brachyurus* and *P. neglectus* were distributed sporadically, with detection rates up to 10%, 3%, and 2%, respectively. A single-step multiplex PCR was developed for the simultaneous detection of *P. scribneri*, *P. crenatus*, and *P. penetrans*. Sequence data from this research and NCBI were used to generate different primer sets that are species-specific. We have designed 24 and selected 3 sets of primers that discriminate *P. scribneri*, *P. crenatus*, and *P. penetrans* in multiplex PCR. All the tested primers showed specificity and had no cross-reaction with non-tagret species. When used in a uniplex, duplex, and triplex PCR, the 3 selected primers gave a unique electrophoretic DNA banding pattern characterized by a single DNA fragment for *P. scribneri* (ca. 750), *P. crenatus* (ca. 690), and *P. penetrans* (ca. 520). The method could be used for routine diagnostic programs.

Competitive ability of iprovalicarb-resistant mutants of *Phytophthora capsici*

X. H. LU (1), J. Hao (2), X. Liu (1)
(1) China Agricultural University, Beijing, PRC PEOPLES REP OF CHINA; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S74

Iprovalicarb is a compound that inhibits plant pathogenic oomycetes, but its application may be limited by resistance in the pathogen. Iprovalicarb-resistant mutants of *Phytophthora capsici* were developed in the laboratory, and four of them (R2-1, R2-2, R1-3, and R1-7) were used to evaluate their competitiveness. Zoospore suspensions of resistant and sensitive isolates were mixed at ratios of 1:9, 3:7, 5:5, 7:3 and 9:1, respectively, inoculated on Petri plates containing carrot agar (CA), and incubated at 25°C. After production of sporangia, zoospore suspensions were prepared and spread on CA medium amended with or without iprovalicarb (5 µg/ml), to determine the frequency of iprovalicarb-resistant subpopulation. Zoospores were concomitantly transferred to CA plates to initiate a new cycle of growth. After five cycles,

R2-1 and R2-2 were nearly non-detectable, but R1-3 and R1-7 were dominant. In the greenhouse, sweet pepper (*Capsicum frutescens*) seedlings were inoculated by pouring zoospore suspensions in the rhizosphere. After three days, *P. capsici* was isolated from the stem tissues of 20 infected seedlings, assayed for competitiveness, and inoculated on plants for five cycles of infection. Regardless of initial ratios of the resistant to sensitive isolate, R2-1 and R2-2 were dominant, and R1-3 and R1-7 were less competitive than the sensitive isolates at last. Thus, some iprovalicarb-resistant isolates may survive competitively under contain conditions.

Immunodiagnostic assays targeted to urediniospore wall proteins of Asian soybean rust

D. G. LUSTER (1), M. B. McMahon (1)
(1) USDA ARS Foreign Disease Weed Science Research Unit, Fort Detrick, MD, U.S.A.
Phytopathology 100:S75

Phakopsora pachyrhizi, the causal agent of Asian soybean rust (ASR), continues to expand across the southeast and mid-south regions of the U.S., resulting in increased fungicide applications for producers. Our objectives in this research were to identify ASR protein targets for development of immunodiagnostic assays, preferably expressed *in planta* and early in infection. We identified and characterized a small family of extracellular proteins in the *P. pachyrhizi* urediniospore wall, termed PHEPs (for PHakopsora Extracellular Protein). One protein family member, PHEP 369, was selected as an ideal immunodiagnostic target after localization studies confirmed its extracellular location and Western blot analysis detected PHEP 369 in plants as early as 3 DPI. Monoclonal antibodies (MAbs 2E8E5-1 and 3G6H7-3) generated against recombinant PHEP 369 were tested for sensitivity against the recombinant protein and extracts from ASR-infected plants, and for specificity against a set of common soybean pathogens (from cultures and infected soybeans). MAb 3G6H7-3 was highly specific for the target protein, detected as little as 10 ng protein, and did not react with any of the soybean pathogens or urediniospores of related rust fungi. Immunolocalization studies with MAb 3G6H7-3 confirmed the urediniospore wall location for PHEP 369. These antibodies will prove applicable in immunodiagnostic assays with infected soybeans and to identify ASR spores from sentinel surveillance plots.

Quantify changes in root architecture caused by *Meloidogyne incognita*, *Thielaviopsis basicola* or their combination on cotton

J. MA (1), J. Jaraba-Navas (1), T. Kirkpatrick (2), C. Rothrock (1)
(1) University of Arkansas, Fayetteville, Fayetteville, AR, U.S.A.; (2) Southwest Research & Extension Center (SWREC), University of Arkansas, Hope, AR, U.S.A.
Phytopathology 100:S75

Cotton root system morphology was evaluated in relation to infection by two soilborne pathogens in controlled environmental studies. *Meloidogyne incognita*, the root-knot nematode, and *Thielaviopsis basicola*, a fungus that causes black root rot, are important cotton pathogens in Arkansas. Both pathogens cause distinct symptoms on affected roots and a synergistic interaction between these two pathogens increases disease losses dramatically. This study characterized the changes in seedling root architecture caused by these pathogens individually and in combination. Cotton was planted in soil either not infested or infested with each of these pathogens or with both pathogens, and placed in growth chambers for six weeks. WinRhizo software was used to determine the morphometric and topological changes including magnitude (number of exterior links), altitude (the longest individual path length) and total exterior path length. Root volumes per plant were 0.631 cm^3, 0.411 cm^3 and 0.219 cm^3 for soil infested with *M. incognita*, *T. basicola* or both pathogens, respectively. Changes in root system morphological and topological parameters caused by either of these pathogens included reductions in root system magnitude, altitude, number of root links and total exterior path length compared to healthy root systems. Analysis of topological parameters to evaluate root system architecture of cotton should enable the quantitative assessment of the effect of root pathogens.

Exploring lineage-specific chromosomes in *F. oxysporum* species complex

L. MA (1), S. Zhou (2), L. R. Gale (3), A. Breakspear (3), A. Chakrabarti (4), D. Gardiner (5), W. Jonkers (3), K. Kazan (5), J. Manners (5), P. Dodds (6), D. Schwartz (2), J. White (1), M. Koehrsen (1), Q. Zeng (1), J. Galagan (1), C. Cuomo (1), J. Ellis (6), C. Kistler (3)
(1) Broad Institute, Cambridge, MA, U.S.A.; (2) Madison, WI, U.S.A.; (3) St. Paul, MN, U.S.A.; (4) Canberra, AUSTRALIA; (5) Brisbane, AUSTRALIA; (6) Black Mountain, AUSTRALIA
Phytopathology 100:S75

The Fusarium comparative genomes of *F. graminearum* (*Fg*), *F. verticillioides* (*Fv*) and *F. oxysporum* (*Fo*) revealed greatly expanded lineage-specific (LS) chromosomes in *Fo*. These mobile LS chromosomes contribute to fungal pathognicity and host–especificity, providing an explanation for the polyphyletic 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) examine genome structural variation and confirm the presence of LS chromosomes among different isolates using optical mapping; 2) determine gene content variation among these selected isolates using next-generation sequencing (NGS); 3) identify all lineage-specific genes using targeted sequencing of the LS chromosomes and RNA sequencing via whole transcriptome approaches. One human isolate and 11 plant pathogenic isolates that represent eight *formae speciales* were included in the study. Preliminary results from the optical mapping confirm the existence of LS chromosomes in different isolates. Genomic data generated using NGS detects genome-wide patterns of mutation among isolates during their brief time of evolutionary divergence. RNA-seq data shows great promise in detecting novel genes encoded in the LS chromosomes and for determining gene expression profiles under different conditions.

Development, registration and commercialization of a microbial fungicide for controlling cotton verticillium wilt in China

P. MA (1), S. Li (1), X. Lu (1), Q. Guo (1), B. Li (1)
(1) Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding, Hebei, PRC PEOPLES REP OF CHINA
Phytopathology 100:S75

Cotton verticillium wilt (CVW), causing by Verticillium dahliae, is a constraint factor in cotton production around the world. Antagonistic Bacillus subtilis strain NCD-2 against V. dahliae was isolated from the cotton rhizosphere in Hebei province of China. The efficacy bioassays of strain NCD-2 for controlling CVW were performed in greenhouse and field conditions. A preparation of the microbial fungicide, 109 cfu/g B. subtilis WP, was formulated with strain NCD-2. Field trials showed that the preparation could reduce 60%–80% severity of CVW by seed treatment in different regions of China. It was no-pathogenic to 8 crops including cotton, wheat, corn, cotton, potato, eggplant, cucumber and soybean. The survival rate of strain NCD-2 was over 90% after 18 months storage under normal condition. This preparation was registered for controlling CVW in China in 2006. The mass production technology of strain NCD-2 was optimized in 500L, 5000L and 15000L fermentation tanks, respectively. Control spectrum studies revealed that the microbial fungicide significantly controlled some other soil born diseases such as eggplant verticillium wilt, cotton fusarium wilt, watermelon fusarium wilt, yam root rot. Preliminary study indicated that the supposed action mechanisms of strain NCD-2 included inhibition (antifungal compounds production), colonization on the surface of cotton root and transportation in the cotton plant to block the pathogen extension, and growth promotion.

Demethylation inhibitor (DMI) fungicide resistance mechanism in *Sclerotinia homoeocarpa* causing dollar spot in turfgrasses

B. MA (1), L. P. Tredway (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S75

The cytochrome P450 sterol 14α-demethylase gene (ShCYP51) in *Sclerotinia homoeocarpa* was cloned and sequenced. ShCYP51 gene was 1680 bp in length and contained two introns of 53 bp and 57 bp located after nucleotide positions in 247 and 498, respectively. The deduced amino acid sequence had a similarity of 73.0, 70.5 and 54.5% to the CYP51 genes from *Monilinia fructicola*, *Botryotinia fuckeliana*, and *Venturia inaequalis*, respectively. An ~1700 bp fragment in the upstream region of the gene was also amplified and sequenced. It was predicted to have two promoters located at the base pair position of -122 to -172 and -791 to -841 of the gene, respectively. The sensitivities of 43 *S. homoeocarpa* isolates to DMI fungicide were determined *in vitro* using a discriminatory dose of 0.02 μg/ml of propiconazole. The ShCYP51 genes and the promoter regions from those isolates were sequenced and compared. Three types of point mutation were detected among 12 isolates; however, none of them were associated with DMI sensitivity. No inserts or repeats was found in the promoter region from any of the isolates. Real-time PCR was used to quantify ShCYP51 gene expression in six sensitive and six resistant isolates, and no difference was found between the two types of isolates. Results from this study show that point mutation and over-expression of the ShCYP51 gene and modifications of the gene's promoter region are not the mechanisms operating in *S. homoeocarpa* leading to the resistance to DMI fungicide.

Molecular characterization of Colletotrichum populations causing crown rot of strawberry in Australia

S. Mackenzie (1), A. Gomez (2), D. Hutton (2), N. A. PERES (1)
(1) University of Florida, Gulf Coast Research and Educaion Center, Wimauma, FL, U.S.A.; (2) Queensland Primary Industries and Fisheries, Maroochy Research Station, Nambour, AUSTRALIA
Phytopathology 100:S75

Colletotrichum crown rot of strawberry is caused by two closely related species, *C. gloeosporioides* and *C. fragariae*. Within the *C. gloeosporioides* species designation, two subpopulations exist in the U.S. One heterothallic and highly variable that is commonly found on strawberry and other hosts, and one homothallic that is often referred to as *Glomerella cingulata*. *C. fragariae* was believed to occur only on strawberry, but we recently reported affecting other hosts. Colletotrichum isolates from crown rot-affected plants in Australia were compared to isolates from the U.S. using sequence data from three areas of the genome. Among the *C. gloeosporioides* isolates, only two different genotypes were observed in Australia, in contrast to the highly variable population from the U.S. Most isolates sampled were from a homothallic subpopulation that more appropriately should be described as *G. cingulata*. This subpopulation was not found on strawberry in the U.S. Only a couple of *C. gloeosporioides* isolates was found in Australia that are probably from the same subpopulation commonly isolated from diseased crowns in the southeastern U.S. *C. fragariae* isolates from Australia were genetically the same as an isolate found on date palm, but not strawberry, in the U.S. The lack of genetic diversity of the populations in Australia may indicate that the fungus is spread mainly on transplants and does not come primarily from alternate hosts.

Morphological and pathogenic variability of *Cochliobolus sativus* from Nepal

B. N. MAHTO (1), S. Gurung (1), T. B. Adhikari (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S76

Spot blotch, caused by *Cochliobolus sativus*, is a major disease of wheat in Nepal. A total of 48 monoconidial cultures of *C. sativus*, collected from different locations in Nepal, were analyzed for morphological characteristics and variation in virulence on wheat genotypes. Among them, 11 isolates (CS 2, 9, 10, 12, 15, 17, 19, 24, 33, 37, and 45) varied from each other in colony appearance such as color of the substrate, margin, zonation, sectoring, and exudation. The eleven isolates plus isolates CS 32 and 49 were further tested for virulence on eight wheat genotypes (Chirya 1, Chirya 7, Milan/Shanghai 7, SW 89-5422, PBW 343, BL 1473, BL 3036, and RR 21) at the seedling stage in a greenhouse at NDSU. Based on infection responses, wheat genotypes varied in resistance and susceptibility. The wheat cultivar Chirya 7 was resistant to all isolates tested. *C. sativus* isolates also differed significantly from each other in virulence. For example, isolate CS 45 from Bhairahwa was highly virulent followed by CS 49, 33, and 37, while isolate CS 12 from Bhaktapur was the least virulent. In general, isolates from the Tarai (plain) region were more virulent than isolates from higher elevation. The interaction between wheat genotypes and isolates was significant, indicating the possibility of race specificity.

Spatial heterogeneity of leaf wetness duration in winter wheat canopy and its influence on plant disease epidemiology

A. Mahtour (1), M. El Jarroudi (1), F. Giraud (2), P. Delfosse (3), L. Huber (4), L. Hoffmann (3), B. TYCHON (1)
(1) Arlon, BELGIUM; (2) Martillac, FRANCE; (3) Belvaux, LUXEMBOURG; (4) Grignon, FRANCE
Phytopathology 100:S76

Leaf wetness duration (LWD) is an important factor influencing the occurrence of plant disease epidemiology. Despite considerable efforts to determine LWD, little attention has been given to study its variability within the canopy. The objective of this study was to evaluate its spatiotemporal variability in wheat fields in a heterogeneous landscape. The spatiotemporal variability of LWD was evaluated in a site close to Arlon (Belgium) during the period May to July 2006 and 2007. LWD measurements were made using a set of flat plate sensors deployed at five different distances from a 18 m high hedge (5, 10, 20, 50, 100 m). Each set of two sensors was placed horizontally close the flag leaf. In addition, we collected the amount of dew water that deposited on rigid epoxy plates placed next to each sensors. Experimental results showed that LWD measurements revealed substantial heterogeneity among sensor positions. LWD is longer for sensors closer to the hedge mainly because of its shadowing effect. 3 to 4 hours of difference was observed between sensors located at 5 m and those located at 100 m, and besides, a significant quantitative difference ($p < 0.0001$) of dew deposit was observed between area beside hedge and those placed at 100 m. In summary, this study provides new information on how wetness is distributed on wheat leaves according to the distance from a hedge. This leads to local microclimate conditions that will contribute to the disease spatial heterogeneity.

Development of prototype Pathogen Detection Lab-On-a-Chip (PADLOC) system for real-time on-field plant disease diagnostics

M. M. MALAPI-NELSON (1), V. L. Vaughn (1), B. Ma (1), A. Han (1), D. Gross (1), W. Shim (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S76

Crop production in the U.S. is highly vulnerable to new and emerging diseases. The introduction of exotic plant pathogens is continually challenging U.S. agriculture and today threatens a variety of production fields. Consequently, there is a critical need in developing new technologies for rapid and accurate detection and identification of these pathogens. In particular, implementation of portable and rapid diagnostics tool can facilitate effective on-field plant pathogen detection. To achieve this aim, we are developing a real-time PCR microchip that will engine the portable plant pathogen detection lab-on-a-chip (PADLOC) system. We are constructing a microsystem composed of a microfluidic chamber, where PCR occurs, and integrated microfabricated electrodes as heater and temperature sensor. PCR volume (2 µl) in this chamber enables rapid heating and cooling. A computer controlled instrument controls PCR, and a compact fluorescent detection unit allows real-time PCR monitoring. Also, we are currently optimizing sample preparation and micro-scale real-time PCR protocol methodologies necessary to achieve high diagnostic specificity and sensitivity in PADLOC system. Specifically, we are using *Pseudomonas syringae* pv. *syringae* B728a and *Fusarium oxysporum* f. sp. *lycopersici* as models that represent plant bacterial and fungal pathogens, respectively, to develop on-field pathogen DNA extraction protocols as well as specific PCR diagnostic assays.

Intraspecific analysis of *Phytophthora nicotianae* from diverse hosts and geographic locations using mitochondrial and nuclear markers

M. A. MAMMELLA (1), L. Schena (2), M. D. Coffey (3), S. O. Cacciola (4), F. N. Martin (5)
(1) Dipartimento Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, Italy; USDA-ARS, Salinas, CA, U.S.A.; (2) Dipartimento Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, ITALY; (3) Department of Plant Pathology and Microbiology, University of California, Riverside, CA, U.S.A.; (4) Dipartimento di Chimica biologica, Chimica medica e Biologia molecolare, Università degli Studi di Catania, ITALY; (5) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 100:S76

Phytophthora nicotianae is an economically important pathogen with a worldwide distribution that causes disease in hundreds of plant species. In Italy it is a particular problem on citrus, where it primarily causes root rot. In an effort to better understand the population structure of isolates recovered from citrus in Italy and how these relate to those recovered from different hosts and geographic regions we have been examining the mitochondrial haplotype for a collection of over 90 isolates. Four regions of the mitochondrial genome (totaling 3 kb) have been sequenced for this purpose with a total of 49 haplotypes identified thus far. Interestingly 17 isolates from citrus recovered from Italy, California and Philippines represent 9 haplotypes that cluster closely together (these are differentiated by a total of 9 SNPs and 5 deletions occurring in two homopolymeric thymine regions). Single citrus isolates from Tunisia, Trinidad and Italy have distinctly different haplotypes. Some isolates from other host species exhibited a similar type of grouping, with many isolates recovered from tobacco and ornamentals clustering in their individual host associated clades. Investigations classifying nuclear genotypes for the same group of isolates are currently under way and their correlation with mitochondrial haplotypes will be discussed.

Direct delivery of viral vectors into plant suspension cells

S. A. MANABAYEVA (1), H. B. Scholthof (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S76

Plant cell suspension cultures are of great interest for the biotechnology industry to synchronously produce valuable proteins on a large scale. For this purpose we developed *Tomato bushy stunt virus* (TBSV) DNA- based expression vectors for direct delivery into plant suspension cells. As expression platforms we used a previously established *Nicotiana tabacum* (NT1) suspension cell culture and generated a new culture from *N. benthamiana* (NB). NT1 and NB suspension cells were transformed using biolistic and *Agrobacterium*-mediated gene delivery with TBSV DNA-cassettes expressing GFP. Biolistic delivery was performed at 9 cm from stopping screen to target tissue using DNA coated gold particles, at 1350 psi helium pressure. *Agrobacterium*-mediated transformation parameters that continue to be optimized include different strains, bacterial concentrations, incubation and co-cultivation periods, and acetosyringone concentrations. Initial results showed that with both delivery systems, transient GFP expression is observed within a few days post-transformation. In conclusion, we established a new cell suspension culture of *N. benthamiana*, and adapted biolistic and *Agrobacterium*–mediated delivery systems for newly developed TBSV-based DNA vectors into cell suspension cultures.

Alternative method for rapid processing, shipping and testing of a large number of psyllids for the presence of "*Candidatus* Liberibacter spp."

K. L. MANJUNATH (1), C. Ramadugu (2), J. Drake (1), H. A. Arevalo (3), S. E. Halbert (4), P. A. Stansly (3), R. Lee (1)
(1) USDA ARS National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, U.S.A.; (2) Dept. of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, U.S.A.; (3) University of Florida SWFREC, Immokalee, FL, U.S.A.; (4) Florida Division of Plant Industry, Gainesville, FL, U.S.A.
Phytopathology 100:S77

Huanglongbing is a devastating disease of citrus in several countries. HLB associated "*Candidatus* Liberibacter spp." can be detected in psyllids (*Diaphorina citri*) long before the development of symptoms in trees. Currently, psyllids are being monitored for the presence of Liberibacters in several citrus industries for both prevention and management of HLB. Hand-collected psyllids are preserved in ethanol, shipped to testing laboratories, stored in freezers, DNA is then extracted by using standard methods and the presence of Liberibacters is tested by real time PCR. A simple alternative method for rapid processing of a large number of samples was developed in this study initially using another psyllid, *Bactericera cockerelli* carrying "*Ca*. L. psyllaurous" associated with psyllid yellows of tomatoes. The procedure was then successfully applied to the Asian citrus psyllids. DNA is fixed by pressing the frozen insects between two layers of a cellulose membrane. The psyllid remains are then removed, and the membranes are shipped to testing laboratories at ambient temperature. DNA was released from the membranes by boiling them in an extraction buffer before testing by real time PCR. This alternative method was found to be comparable in the sensitivity of detection to standard protocols currently being followed, but allows rapid processing of a large number of samples at significantly lower cost.

Identification of *Burkholderia* sp. genes related to biological control of phytopathogens

E. T. Mano (1), A. A. Neves (1), V. C. Santos (2), A. FERREIRA (2), W. L. Araújo (1)
(1) Laboratory of Molecular Biology and Microbial Ecology, NIB, University of Mogi das Cruzes, Mogi das Cruzes, SP, BRAZIL; (2) Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, BRAZIL
Phytopathology 100:S77

The species from *Burkholderia* genus have been considered important target for molecular studies mainly due the potential for biological control of phytopathogens. These species are able to produce a huge variety of antimicrobial compounds, which have been biochemically characterized. However, the genes associated to the synthesis of these molecules are poorly described, mainly due the low number of genetic studies in this area. In this context, molecular techniques have been applied to identification and genetic characterization of this pathway. Therefore, the aim of this work was the identification of genes from endophytic bacterium *Burkholderia* sp. associated to biocontrol of *Pectobacterium carotovora* in Orchids and biocontrol *in vitro* of fungal *Fusarium oxysporum*, *Fusarium verticillioides*, *Ceratocystis paradoxa*, *Colletotrichum* sp., and the *Phytophthora parasitica*. For this, a library with 1788 clone was obtained by random mutagenesis based on Tn5 transposon insertion. Mutants were confirmed by specific PCR for Tn5 transposon and hybridization with specific probes confirmed the number of Tn5 insertion. Clones defectives to control of these pathogens or showing a variation in this phenotype were selected. Some mutants showed different growth rate and colony pigmentation. The analysis of partial sequence of 15 DNA sequences showed that 40% is *hypothetical protein*, allowing the association of some phenotypes to these putative genes. The identification and cloning of such genes will allow a better understanding of the production of these antimicrobial compounds and further applying in a biotechnological view.

***Methyl esterase 1* (*StMES1*) is required for systemic acquired resistance against *Phytophthora infestans* in potato**

P. MANOSALVA (1), S. Park (2), F. Forouhar (3), L. Tong (4), W. Fry (5), D. Klessig (1)
(1) Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.; (2) Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA, U.S.A.; (3) Department of Biological Sciences, Northeast Structural Genomics Consortium, Columbia University, New York, NY, U.S.A.; (4) Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, New York, NY, U.S.A.; (5) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S77

In tobacco and Arabidopsis, methyl salicylate (MeSA) serves as a long-distance phloem-mobile signal that must be converted into its biologically active form, salicylic acid (SA), in the distal uninfected tissue by the esterase activity of salicylic acid-binding protein 2 (SABP2) in tobacco or members of the AtMES family in Arabidopsis. In contrast to tobacco and Arabidopsis, which have very low levels of endogenous SA, potato contains a high basal level of SA and its role in the development of systemic acquired resistance (SAR) has been controversial. In this study we identified the potato ortholog of tobacco SABP2 (StMES1) and showed that the recombinant protein shares similar biochemical properties with NtSABP2. Recombinant StMES1 converts MeSA to SA and its esterase activity is feedback inhibited by SA or its synthetic analog, 2,2,2,2'-tetra-fluoroacetophenone (tetraFA). Moreover, potato in which the distal uninfected tissue was treated with tetraFA was compromised for the development of SAR induced by arachidonic acid (AA) against *Phytophthora infestans*. Similar results were obtained via a genetic approach; *StMES1*-silenced potato displayed a defective SAR phenotype that correlated with elevated MeSA levels in the uninfected distal tissue. In addition, AA-induced *pathogenesis-related* (*PR*) gene induction was attenuated in these plants as compared with the wild type. Together these findings argue that StMES1, and MeSA are critical components for SAR in potato.

Using confocal microscopy to study the infection of *Mentha longifolia* by a GFP strain of the verticillium wilt pathogen

M. E. MANSFIELD (1), K. J. Vining (2), M. A. Townley (3), T. M. Davis (4)
(1) Microbiology B.S. Program, University of New Hampshire, Durham, NH, U.S.A.; (2) Oregon State University, Dept. of Forest Ecosystems and Society; also Oregon State University Center for Genome Research and Biocomputing, Corvallis, OR, U.S.A.; (3) Department of Zoology, University of New Hampshire, Durham, NH, U.S.A.; (4) Department of Biological Sciences, University of New Hampshire, Durham, NH, U.S.A.
Phytopathology 100:S77

The threat of verticillium wilt, caused by *Verticillium dahliae*, is a serious concern of the U.S. mint industry. Peppermint (*Mentha* × *piperita*) is susceptible to the wilt fungus, while spearmints (*M. spicata*, *M. gracilis*) are less susceptible to infection. Commercial mint species are polyploid, presenting difficulties as genetic research subjects. Therefore we are studying the related diploid species, *Mentha longifolia*, with the aim of identifying genes that may act to confer tolerance to *Verticillium* infection in mint. A GFP strain of *V. dahliae* obtained from Lynda Ciuffetti at Oregon State University was used to infect tolerant and susceptible *M. longifolia* accessions. Confocal microscopy was used to track the fungal invasion and dissemination within the host plant. The progression of the infection in root and vascular tissues was examined at two day intervals post-inoculation. Equally extensive root infection was observed in both the susceptible and the resistant accessions of mint. By eight days post-inoculation, the fungus was observed in stem tissue of susceptible plants. Guided by these findings, we targeted infected stem tissue for total RNA isolation, the sequencing of which will provide an opportunity to examine patterns of plant and fungal gene expression during the infection process. This knowledge may help identify plant genes that confer tolerance to *V. dahliae* infection.

Multiplex PCR for simultaneous detection of eight major onion bacterial pathogens

M. Mansfield (1), B. GUGINO (1)
(1) The Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S77

Bacterial diseases of onion cause significant pre- and post-harvest yield losses in Pennsylvania and other onion growing regions in the U.S. Correlating symptoms to specific pathogens using traditional morphological and physiological techniques is both labor and time intensive and often confounded by isolate variation. To facilitate rapid detection of these bacterial pathogens from onion tissue, we have developed a multiplex PCR method to simultaneously detect *Burkholderia cepacia*, *B. gladioli*, *Pectobacterium caratovora*, *Pantoea ananatis*, *P. agglomerans*, *Pseudomonas marginalis*, *P. viridiflora*, and *Xanthomonas axonopodis*. Eight sets of species specific primers, based on the single copy gyrase B gene, were used in a multiplex PCR reaction and the resulting PCR products resolved using standard gel electrophoresis. Pathogen detection from symptomatic bulb and leaf tissue using the multiplex PCR was consistently more sensitive and reliable than culturing with selective media, thus the sample size and statistical power of research trials can be increased and bacterial diseases in commercial onion fields diagnosed more rapidly and accurately. This method will augment current efforts to study onion bacterial pathogens and assist future work to develop integrated pest management practices for onion in Pennsylvania.

Screening of plant introduction materials from *Brassica species* for resistance against PG3 and PG4 isolates of blackleg

D. A. MARINO (1), L. E. del Rio (1)
(1) Dept. Plant Pathology North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S77

Blackleg, caused by *Leptosphaeria maculans*, is the most destructive pathogen of canola (Brassica napus and *B. rapa*) in North America. Strains of

this pathogen have been classified in pathogenicity groups (PG) depending on their virulence profile on three differential genotypes. The discovery of strains with new virulence profiles (PG3 and PG4) in North Dakota and Western Canada highlights the need to characterize the reaction of commercial cultivars to such strains as well as to identify sources of resistance against them. Efforts are in place to close this gap. Greenhouse trials are being conducted to evaluate the reaction of cultivars and plant introduction materials to multiple strains of PG3 and PG4 using the cotyledon test. In these replicated trials, seedlings were inoculated with a blend of 3–4 isolates of each PG (10^7 spores ml^{-1}) and incubated for 24 hours in a misting chamber. Plants were returned to greenhouse room and evaluated for disease reaction ten days later using a 0–9 scale. Only two of the 72 commercial cultivars were considered moderately susceptible while the rest were susceptible to strains of both PGs. All 450 *B. rapa* accessions were considered susceptible, while seven of 220 *B. juncea* accessions evaluated so far were classified as moderately resistant to both PGs. Seedlings of these materials have been taken to seed production to evaluate their reaction to blackleg as adult plants. Additional *B. juncea* materials are being evaluated.

***Xanthomonas sacchari* – a pathogen or an endophyte?**
C. J. MAROON-LANGO (1), K. L. Schneider (2), R. S. Turner (1), G. G. Presting (2), A. M. Alvarez (3)
(1) USDA APHIS PPQ PHP Plant Germplasm Quarantine Program, Beltsville, MD, U.S.A.; (2) Molecular Biosciences and Bioengineering, University of Hawaii, Manoa, HI, U.S.A.; (3) Plant Pathology Department, University of Hawaii, Manoa, HI, U.S.A.
Phytopathology 100:S78

During routine indexing of sugarcane at the Plant Germplasm Quarantine Program of the USDA-APHIS, colonies typical of xanthomonads were isolated from symptomless sugarcane introductions on selective media routinely used for isolating *Xanthomonas albilineans*, the cause of bacterial leaf scald of sugarcane. Tissue blot assay of cane setts from the same plants using the antiserum to the leaf scald bacterium was negative, indicating that the xanthomonads were distinct from *X. albilineans*. From six of the seven xanthomonad isolates, PCR using the ITS primers for *Xanthomonas* resulted in an amplicon with a 450-bp stretch that shared 100% identities with *X. sacchari*. RIF primers were used to confirm the identity obtained with the ITS. The six isolates grouped basal to *X. albilineans*, however no reference match existed in the database. Because of the rarity by which we encounter *X. sacchari* and the lack of information on its pathogenicity, we are inoculating a few sugarcane introductions, sorghum and Miscanthus. Results of the bioassay will be presented.

Biological traits of *Pectobacterium* clades
M. Marquez-Villavicencio (1), A. CHARKOWSKI (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S78

The reported host range of *Pectobacterium* species includes over 30% of dicot plant families and 60% of monocot plant families. It is often difficult to determine from published reports which *Pectobacterium* species or subspecies was isolated and characterized, thus the host range of the *Pectobacterium* taxa remains obscure. Recently, multi-locus sequence analysis allowed us to place *Pectobacterium* strains into well-supported clades. To test the hypothesis that *Pectobacterium* clades vary in host range, we inoculated previously reported *Pectobacterium* hosts with representative strains from three *Pectobacterium* clades. These clades include the narrow host range pathogen *P. atrosepticum* (Pa), the broad host range pathogen *P. carotovorum* subsp. *carotovorum* (Pcc), and *P. carotovorum* subsp. *brasiliensis* (Pcb), for which the host range is unknown. Our results did not support the hypothesis that Pa is a narrow host range pathogen; some Pa strains infected hosts such as carrot, onion, radish, celery, and rapini. Similarly, Pcc and Pcb strains varied and most were similarly limited in host range as Pa. We were unable to infect corn, spinach, beet, or asparagus with any of the strains tested. We also inoculated tubers and stems of commercial potato varieties to determine if these three bacterial taxa vary in aggressiveness on potato and found that Pcc and Pcb did not differ from each other, but that they both caused significantly more tuber decay, but not more stem decay, on all potato varieties than Pa.

Insights into the introduction of bacterial heart rot of pineapple to Hawaiian plantations on the basis of molecular and biochemical analyses
G. MARRERO (1), W. Kaneshiro Sueno (1), A. S. De Silva (1), A. M. Alvarez (1)
(1) University of Hawaii at Manoa, Honolulu, HI, U.S.A.
Phytopathology 100:S78

Bacterial heart rot disease of pineapple caused by *Dickeya* sp. is a major constraint to pineapple production throughout the tropics and subtropics. In 2003, the disease was reported in Hawaiian fields planted with stocks that had originated from Costa Rica and Honduras. Regulatory action prevented further importation of stocks from these locations and fields with diseased plants were subsequently destroyed. In 2006, an outbreak of the disease was again seen in Hawaiian pineapple fields. Since *Dickeya* sp. can cause latent infections and vegetative materials are used for successive crops, our study aimed at determining the source of the 2006 outbreak. Rep-PCR fingerprint analysis distinguished three groups observed from the 2003 outbreak; these groups were different from those observed from the 2006 strains. Molecular phylogenies produced from *dnaA*, *dnaX*, *dnaJ*, and *recN* sequences confirmed the separation of strains isolated in 2003 and 2006 and supported the hypothesis that two separate introductions of the pathogen gave rise to these outbreaks. Strains isolated in 2006 were more closely related to *Dickeya* sp. reference strains isolated from Malaysia. Although the strains isolated from infected pineapple were grouped together in a single cluster sister to *D. zeae*, these strains differ biochemically and genetically from this species. This group is sufficiently different to warrant classification as a new species.

Mitochondrial haplotype analysis as a tool for differentiating populations of *Verticillium dahliae*
F. N. MARTIN (1)
(1) USDA ARS, Salinas, CA, U.S.A.
Phytopathology 100:S78

The ability to monitor mitochondrial background in *Verticillium dahliae* may provide an additional tool for population studies and monitoring clonal populations. Published mitochondrial genome sequences of *V. dahliae* (DQ351941) were used to design primers for amplification of 5 regions representing 19% of the genome (5.2 kb) for assessment of mitochondrial haplotype. Observed differences among isolates representing a range in VCG, host, and geographic origin were due to single nucleotide polymorphisms, different numbers of bases in specific homopolymeric regions, and copies of subrepeated sequences. For 30 isolates a total of 15 mitochondrial haplotypes were identified. Some of the observed grouping correlated with VCG. For example, five VCG-1A and –1B isolates from California, Spain and Greece had identical haplotypes. While a single haplotype predominated among a group containing VCG-2A isolates, there were 8 haplotypes within a group containing VCG-2B isolates. Likewise, five VCG-4 isolates fell into 4 mitochondrial haplotypes, one of which was identical to the VCG-2A grouping. Phylogenetic analysis with these five regions revealed the mitochondrial background of VCG-1 to be monophyletic but VCG-2 and VCG-4 were polyphyletic. The results obtained indicate that variation in mitochondrial haplotypes may be a useful for characterization of isolates, particularly those which are heterokaryon self-incompatible.

Turfgrass disease identification and management aided by "smart" phone technology
A. MARTINEZ-ESPINOZA (1), C. F. Waltz (1), W. Hudson (2), P. McCullough (1)
(1) University of Georgia, Griffin, GA, U.S.A.; (2) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S78

Turfgrass managers, extension professionals, crop consultants and sod producers often require real-time, *in-situ* disease management recommendations and disease diagnosis. Taking advantage of universal mobile telecommunications systems, remote server technology and downloadable programs, we have developed an application (app) for Iphone® and Blackberry® "smart phones" which allows access to a library of resources in the field. The "Turfgrass Management" application contains a full suite of disease etiology, symptomatology, epidemiology, and disease and pathogen resources. Included are image galleries, cultural and chemical management strategies for forty-three foliar, root, crown, abiotic and non-infectious diseases, and descriptions of more than eighty fungicide products. The interface allows easy searching and cross-referencing by active ingredients or common name of fungicides, or by disease. Information is kept current and readily can be updated for new diseases, cultural strategies or new fungicide products. Additionally, the program contains a comprehensive description of turfgrass broadleaf, grassy, post emergent and pre-emergent weeds, herbicides, growth regulators, insects and insecticides as well as turfgrass taxonomy. More than 1600 subscriptions from over thirty countries have been downloaded in the nine weeks since the program was released.

Influence of temperature and leaf wetness duration on orange rust of sugarcane
T. D. MARTINS (1), R. N. Raid (2), W. L. Burnquist (3), A. S. Urashima (4), A. Bergamin Filho (1), J. Comstock (5)
(1) Escola Superior de Agricultura Luiz de Queiroz / USP, Piracicaba, BRAZIL; (2) Everglades Research and Education Center/ UF, Belle Glade, FL, U.S.A.; (3) Centro de Tecnologia Canavieira, Piracicaba, BRAZIL; (4) Centro de Ciências Agrárias/UFSCar, Araras, BRAZIL; (5) USDA/Sugarcane Field Station, Canal Point, FL, U.S.A.
Phytopathology 100:S78

Sugarcane orange rust, caused by *Puccinia kuehnii*, is a disease of increasing concern. New to the Western Hemisphere since 2007, yield losses in excess of 40% have been reported on susceptible varieties in Florida. Epidemiological studies were performed to investigate the influence of temperature and leaf wetness duration on mean lesion density and disease severity. Tests were carried out at temperatures 10, 15, 20, 25, 30 and 35°C, and leaf wetness durations of 0, 4, 8, 12, 18 and 24 h (moist-chamber conditions), in a combined manner. Inoculations were performed on sugarcane plants, in pots, using the orange rust susceptible variety CL85-1040. All experiments were repeated twice. Spore suspensions were spread on the leaf surfaces and the pots were maintained inside growth chambers. Disease incidence and severity were recorded during every assessment. Significant orange rust symptoms developed over a rather limited temperature range of 20–25°C, and lesions were notably larger at 25°C than at 20°C. An incubation and latent period of 11–17 days were observed at these temperatures. The pathogen required a minimum of 8 h of leaf wetness for successful infection and rust was predictably most severe at leaf wetness durations in excess of 12 h. The prevalence of favorable temperature conditions (20–25°C) and leaf wetness durations (> 8 h) throughout much of the growing season in Florida may help to explain the importance of this newly introduced disease. Research supported by CNPq.

Agrobacterium-mediated transformation is an efficient tool for insertional mutagenesis of the vascular wilt fungus, _V. dahliae_

K. MARUTHACHALAM (1), S. Kang (2), S. J. Klosterman (3), K. V. Subbarao (4)
(1) University of California, Salinas, CA, U.S.A.; (2) Pennsylvania State University, University Park, PA, U.S.A.; (3) USDA ARS, Salinas, CA, U.S.A.; (4) University of California, Davis, CA, U.S.A.
Phytopathology 100:S79

Agrobacterium tumefaciens-mediated transformation (ATMT) is a highly efficient tool for both the targeted and random mutagenesis of filamentous fungi. In this study, ATMT was optimized for Verticillium dahliae, the causal agent of vascular wilt on many economically important crops worldwide. A. tumefaciens strain EHA105, carrying a hygromycin phosphotransferase gene (hph) and a green fluorescent protein (GFP) gene, was used to transform conidia of V. dahliae. The transformation efficiency was correlated with co-cultivation time. Southern blot analysis indicated that T-DNA was inserted randomly into the V. dahliae genome and about 80% of the transformants had a single copy of T-DNA integration. DNA sequences flanking T-DNA insertion were identified through inverse PCR followed by sequencing. Several mutants with altered phenotypes were obtained during the development of mutant library, including those that lost the ability to form microsclerotia. Based on preliminary virulence assay of 130 transformants, we identified about 10 mutants that did not cause any symptoms on lettuce plants. In two of these mutants, T-DNA was inserted in the endoglucase I (VdEg-I) and hydroxyl-methyl glutary-CoA synthase (VdHMGS1) genes, which may be required for plant cell wall degradation and parasitic growth of V. dahliae in the susceptible plants. These results suggest that ATMT can be used to identify genes involved in pathogenicity and other biological functions in V. dahliae.

Variation in the number of sub-repeat sequence in the IGS region identifies isolates of _Verticillium dahliae_ from crucifer hosts

K. MARUTHACHALAM (1), Z. K. Atallah (1), K. V. Subbarao (2)
(1) University of California, Salinas, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.
Phytopathology 100:S79

A large number of fungal species including Verticillium dahliae possess sub-repeat sequences in the intergenic spacer region (IGS). We sequenced the IGS rDNA of 111 isolates of V. dahliae from different hosts and analyzed for sub-repeat motif pattern. A Verticillium-specific sub-repeat motif sequence of 17 nucleotides was found and the number of sub-repeats detected ranged from one to eleven arranged at random. All long and short-spored V. dahliae isolates from cruciferous hosts had an only one sub-repeat motif. The highest number of repeats was found in isolates from marigold, cotton and olive, which included 11 sub-repeat motifs. Additionally, a pair of PCR primers designated VdCr1 and VdCr2 were designed from the IGS rDNA to specifically amplify V. dahliae isolates from crucifers. There was no amplification in V. dahliae isolates from non-crucifer hosts with this primer pair. The difference in number of sub-repeats and specific-primer developed in the IGS rDNA region is thus diagnostic and can be used for rapid identification of V. dahliae isolates from crucifer and non-crucifer hosts.

Comparative efficacy of fungicide application programs alternating among different products for management of powdery mildew on cantaloupe

M. E. MATHERON (1), M. Porchas (1)
(1) University of Arizona, Yuma, AZ, U.S.A.
Phytopathology 100:S79

Powdery mildew on cantaloupe and other melon crops, caused by the fungus *Podosphaera xanthii*, can result in significant yield loss. A field trial was conducted in 2009 to evaluate fungicide application programs that alternate between a highly efficacious fungicide and a product with moderate to low activity. Foliar applications of products were made on 18 May and 2, 9 and 16 June. Powdery mildew was first observed in plots on 28 May and a high level of disease developed on nontreated plants by crop maturity. When applied exclusively throughout the trial, reduction of powdery mildew on the underside of leaves, compared to nontreated plants, was 98% for Procure (triflumizole) and Quintec (quinoxyfen), 74% for Flint (trifloxystrobin), 67% for Cabrio (pyraclostrobin), 58% for Quadris (azoxystrobin), 39% for Kaligreen (potassium bicarbonate), 30% for Topsin-M (thiophanate-methyl) and Sovran (kresoxim-methyl), and 21% for Serenade MAX (*Bacillus subtilis*). In comparison, disease reductions ranging from 81 to 100% were recorded in plots when a highly efficacious fungicide (Procure or Quintec) was alternated with one of the tested products with less activity. The high level of disease control achieved at crop maturity when utilizing these treatment programs demonstrates that lower performing fungicides can be part of treatment programs that expose *Podosphaera xanthii* to more fungicidal modes of action as part of a resistance management program without sacrificing acceptable levels of disease control.

Interactions between _Fusarium virguliforme_ and _Phialophora gregata_ in soybean using greenhouse studies

C. MATTUPALLI (1), P. D. Esker (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 100:S79

Sudden death syndrome (SDS) and Brown stem rot (BSR) are two yield-limiting diseases of soybean. SDS and BSR are caused by the fungal pathogens *Fusarium virguliforme* (*Fv*) and *Phialophora gregata* genotypes A (*Pg*A) and B (*Pg*B) respectively. As soil-borne pathogens, we hypothesize that there is a potential for interactions between them. Two greenhouse trials were conducted to study these potential interactions. The experimental design was a multi-factor randomized complete block with soybean variety (Jack and Williams82) and inoculum (untreated control, *Fv*, *Pg*A, *Pg*B, *Fv*+*Pg*A, *Fv*+*Pg*B, *Pg*A+*Pg*B, *Fv*+*Pg*A+*Pg*B) as the factors. Foliar symptoms were assessed at weekly intervals beginning at first foliar symptoms as an estimate of total infected canopy (0–100%) from which an area under disease progress curve (AUDPC) was calculated. AUDPC was affected by variety and inoculum in both trials. Marginal variety × inoculum interactions ($P = 0.07$) were observed during the first trial only. Jack, a variety supposed to be resistant to *Fv* showed increased disease symptoms when inoculated with *Fv* alone or in combination with *Pg*A or *Pg*B compared to Williams82. A varietal difference ($P = 0.03$) was observed for seed yield during the first trial whereas there was an interaction of variety x inoculum ($P = 0.01$) in the second trial. Results from the two trials suggest some interactions between the pathogens to cause yield losses with a differential effect between the two varieties.

Irrigation water is an unlikely source of inoculum of _Pseudomonas cannabina_ pv. _alisalensis_

S. J. MAUZEY (1), C. T. Bull (2)
(1) California State University Monterey Bay, Seaside, CA, U.S.A.; (2) USDA ARS, Salinas, CA, U.S.A.
Phytopathology 100:S79

Pseudomonas cannabina pv. *alisalensis* causes severe bacterial blight on crucifers across the United States. These experiments examined the potential of irrigation water as a source of inoculum for *P. cannabina* pv. *alisalensis*. Water samples were collected from multiple irrigation reservoirs and sprinklers near infected fields. The samples were tested for the presence of *P. cannabina* pv. *alisalensis* using PCR for detection of coronatine and ethylene biosynthesis genes. *Pseudomonas cannabina* pv. *alisalensis* was not detected in irrigation water samples, however the limit of detection using these methods was high. Survival in water was modeled using a rifampicin resistant strain of *P. cannabina* pv. *alisalensis* and enumeration on antibiotic containing media. Population levels of *P. cannabina* pv. *alisalensis* added to irrigation water dropped significantly within the first few days after inoculation. On average *P. cannabina* pv. *alisalensis* populations dropped from 5.5 log (CFU/ml) at inoculation to below 2.0 log (CFU/ml) after one week. Additionally, the relationship between population levels of *P. cannabina* pv. *alisalensis* spray inoculated onto broccoli raab (*Brassica rapa* subsp. *rapa*) and the resulting level of disease was evaluated. Under greenhouse conditions populations of *P. cannabina* pv. *alisalensis* as low as 2.0 log (CFU/ml) resulted in disease. These results indicate that irrigation water is an unlikely source of inoculum for *P. cannabina* pv. *alisalensis* on crucifers.

Quantifying *Fusarium virguliforme* in soil using SYBR green and Taqman assays

G. C. Mbofung (1), M. Vincent (2), A. Fessehaie (3), M. K. Bhattacharyya (4), L. F. LEANDRO (1)
(1) Dept. of Plant Pathology, Iowa State University, Ames, IA, U.S.A.; (2) Food Science, Iowa State University, Ames, IA, U.S.A.; (3) Seed Science Center, Iowa State University, Ames, IA, U.S.A.; (4) Dept. of Agronomy, Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S80

Fusarium virguliforme is a soilborne fungus that causes sudden death syndrome in soybean. An effective method to specifically quantify the pathogen in soil is needed for epidemiological studies on this disease. Our objective was to develop and compare two real-time PCR assays to detect and quantify *F. virguliforme* inoculum density in soil. SYBR green and TaqMan assays were developed based on sequences of the locus 96 and the FvTox1 gene of *F. virguliforme*. Assay specificity was tested on 58 taxonomically closely related and distant fungal isolates. The sensitivity of the assays was appraised on tenfold serial dilutions of genomic DNA, spore suspensions, and soil spiked with conidia. Applicability was evaluated on soil samples from soybean fields and the greenhouse. Real-time PCR assays detected species-specific DNA sequences only. The detection sensitivity for genomic DNA, spores suspensions, and soil spiked of conidia was 5 pg/µl, 500 spores/ml and 50 spores/g soil, respectively, for the SYBR green assay, and 1 pg/µl, 1000 spores/ml and 1000 spores/g soil, respectively, for the TaqMan assay. *F. virguliforme* inoculum density in the soil samples ranged between 0 and 10^6 conidia per g soil. Both assays were equally sensitive and specific for diagnostic and quantification purposes. The assays can be applied in diagnostic surveys of *F. virguliforme* in soil and for quantification of the pathogen in roots.

Nozzle selection for maximizing brown patch control with fungicides

D. S. MCCALL (1), B. J. Horvath (2), M. A. Cutulle (1), A. E. Nichols (1), V. R. Sykes (2)
(1) Virginia Tech, Blacksburg, VA, U.S.A.; (2) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 100:S80

Brown patch (*Rhicoctonia solani*) is the most common and destructive disease on tall fescue (*Festuca arundinacea*). Because of time and labor constraints, turf managers seek ways to maximize the longevity of fungicide applications. Field studies were conducted in 2008 and 2009 to assess the influence of nozzle type on longevity of control with common fungicides. Plots were 1.8 × 1.8 m and were arranged as a randomized complete block design with four replications. Fungicides evaluated included azoxystrobin (0.305 kg ai/ha), polyoxin-D (0.308 kg ai/ha, and a pre-formulation of triadimefon (1.53 kg ai/ha) and trifloxystrobin (0.305 kg ai/ha). Nozzles tested were XR TeeJet, TurfJet, Air Induction TeeJet, and Turbo TwinJet. All fungicides were applied at spray volume of 435 liters per ha. Disease was assessed weekly by visual estimation of brown patch percentage within plots for at least six weeks. In three of five field trials, there were no significant differences between nozzle type. In two trials, TurfJet nozzles had significantly more disease than all other nozzles at three weeks after application. Results indicate that control may be extended slightly using a flat-fan type nozzle instead of flood-jet type.

The σ factor *rpo*N is required by *Brenneria rubrifaciens* for HR elicitation in tobacco and virulence on walnut plants

A. MCCLEAN (1)
(1) CPGRU USDA-ARS, Davis, CA, U.S.A.
Phytopathology 100:S80

Deep bark canker (DBC) of walnut is caused by the bacterium, *Brenneria rubrifaciens*. The disease leads to a chronic reduction in nut yield and tree vigor. DBC symptoms are characterized by deep vertical cracks in trunks and scaffold branches which exude a discolored bacteria laden sap. The disease develops on trees at least 10 years old but is never observed on seedlings. A collection of 650 *B. rubrifaciens* 6D370 transposon mutants was screened for production of the unique red pigment rubrifacine hypothesized to be associated with virulence. Three hyper pigment producing mutants and 81 other pigment producing and pigment deficient mutants were screened in a tobacco leaf bioassay to evaluate hypersensitive response (HR) elicitation. Three of the 84 mutants, including one of the enhanced pigment producers, failed to elicit a HR in tobacco leaves. Two of the three HR minus mutants, Br-212 and Br-415, were attenuated in their ability to cause necrosis on tissue cultured walnut plants with Br-415 being severely attenuated. Genetic analysis revealed Br-415 contained a transposon insertion in an open reading frame (ORF) with homology to *rpo*N-like σ54 factors for RNA II polymerase. Mutant Br-415 also grew slower in minimal medium and was impaired in movement on motility agar relative to wildtype. These findings indicate *rpo*N σ54 dependent transcription is required by *B. rubrifaciens* for HR elicitation in tobacco and virulence on walnuts.

The Fungal Genetics Stock Center at UMKC is an international Biological Resource Center

K. MCCLUSKEY (1), A. Wiest (1), M. Plamann (1)
(1) University of Missouri-KC, Kansas City, MO, U.S.A.
Phytopathology 100:S80

Founded in 1960, the Fungal Genetics Stock Center enters it's fiftieth year of operation during a period of tremendous growth. The collection has more than doubled since moving to UMKC in 2004 and has added new materials that reach out beyond it's traditional constiuancy. Joining our extensive set of genetically engineered Magnaporthe strains are deletion sets for Neurospora, Cryptococcus and Candida as well as molecular genetic tools for working with plant pathogens, industrial fungi, model organisms, and human pathogens. With distribution growing every year, the FGSC sends materials to scientists in over 35 countries every year; approximately half of our orders are from within the U.S. In addition to being part of an NIH funded multi-instituition Functional Genomics Program for Neurospora, the FGSC is involved in cutting edge genomics research with collaborators at the US DOE Joint Genome Institute. The FGSC and its staff are actively involved in national and international societies and ad hoc working groups fostering the development of collection resources.

Management of powdery mildew in cucurbit crops with biopesticides and integrated programs

M. MCGRATH (1)
(1) Cornell University, Riverhead, NY, U.S.A.
Phytopathology 100:S80

Two biopesticides, Organocide (5% sesame oil) and Milstop (85% potassium bicarbonate), were evaluated for powdery mildew in adjacent field experiments with pumpkin, muskmelon, and butternut squash conducted in 2007 and 2009. They were tested alone or combined with host plant resistance and/or conventional, mobile fungicides (Quintec, Procure, and/or Pristine) in various programs with these fungicides applied in alternation a total of 2 to 6 times. Applications were made with a tractor-sprayer on a 7-day interval beginning when symptoms were first found. Both biopesticides usually were effective, especially when used in an integrated program. For example, severity of powdery mildew on upper leaf surfaces was reduced 39% with Milstop and 57% with Organocide applied to a susceptible pumpkin cultivar based on AUDPC values relative to the nontreated susceptible cultivar; severity was reduced 93% and 73% with Milstop and Organocide, respectively applied to a resistant cultivar; and 63–95% with Organocide applied with mobile fungicides to the susceptible and resistant cultivars. All treatments were equally, highly effective (no significant differences) applied to butternut squash (64–100% control) and to muskmelon (96–100% control) based on severity on 8 Sep 2009. In 2007, severity on upper leaf surfaces was reduced 23% with Milstop applied to a susceptible pumpkin cultivar and 61% with winter squash. The reduction was 52% and 89% with Organocide, respectively.

Microarray characterization of the HrpL regulon of the fire blight pathogen *Erwinia amylovora*

R. R. MCNALLY (1), Y. Zhao (2), I. K. Toth (3), P. J. Cock (3), P. E. Hedley (3), G. W. Sundin (4)
(1) Michigan State University, East Lansing, MI, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.; (3) Scottish Crop Research Institute, Invergowrie, SCOTLAND; (4) Scottish Crop Research Institute, East Lansing, MI, U.S.A.
Phytopathology 100:S80

The bacterium *Erwinia amylovora* causes fire blight of apple, pear, and other rosaceous plants. HrpL, an alternate sigma factor recognizing hypersensitive and pathogenicity (hrp) promoters, is required for pathogenesis. Hrp promoters regulate the expression of both structural and translocated components of the type III secretion system (T3SS) in *E. amylovora*. While HrpL plays a vital role in T3SS regulation, HrpL may also influence additional signaling networks controlling other virulence factors. To explore the HrpL regulon of *E. amylovora* Ea1189, an open reading frame-specific whole-genome microarray was constructed. Microarray analysis compared mRNA extracted from wild-type and Δ*hrpL* strains incubated in hrp-inducing minimal medium. Twenty-four genes were found to exhibit fold-change expression ratios greater than 1.5 in Ea1189Δ*hrpL*. Five genes were up-regulated while 19 genes were found to be down-regulated, suggesting positive control by HrpL. Many genes identified have known roles in T3SS while others encode proteins of unknown function. Knockout mutants of genes of interest identified in the microarray experiment were phenotypically analyzed revealing attenuated virulence in an immature pear assay and altered motility. Continued characterization is expected to reveal novel insights into the pathogenesis of fire blight by *E. amylovora*.

Probability distributions for disease severity and time-to-event data
N. MCROBERTS (1), L. V. Madden (2)
(1) Scottish Agric College, Edinburgh, UNITED KINGDOM; (2) OARDC Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S81

When disease severity is measured either as the proportion (or percentage) of tissue diseased, or by using a continuous rating scale, the resulting data are continuous and bounded, and may often be skewed. Until now the fact that such data do not have strictly Gaussian (normal) properties has usually been ignored in plant pathology, and statistical analyses founded on assumptions of normality are applied routinely. With advances in generalized nonlinear and mixed modeling algorithms it is now straightforward to fit probability distributions with appropriate statistical properties to severity data. We illustrate two examples of such distribution: the beta distribution and Johnson's SB distribution. Changes in the parameters of these distributions over time characterize changes in the spatial heterogeneity of severity during disease progress; preliminary analysis of several diverse data sets indicates a common relationship between the skewness and kurtosis of observed data irrespective of measurement scale of visual symptoms. In the case of disease incidence data, we show that the Laplace distribution provides an alternative means to describe disease progress over time. The cumulative distribution function serves as a reasonable time-to-event model for disease progress, with the corresponding probability density function describing the instantaneous rate of disease progress.

Characterization of nepoviruses by an integrated approach
E. MEEKES (1), M. Botermans (2), R. Hooftman (1), T. van Schadewijk (3), R. Miglino (3), A. Roenhorst (2)
(1) Naktuinbouw, Roelofarendsveen, NETHERLANDS; (2) Plant Protection Service, Wageningen, NETHERLANDS; (3) Dutch Flower Bulb Inspection Service, Lisse, NETHERLANDS
Phytopathology 100:S81

In 2006 a five year program started to reinforce plant health infrastructure in the Netherlands. This program focused on regulated organisms, aims to update existing plant-pathogen collections, enables the development of new detection methods and to share data in an innovative way. Included isolates are characterized biologically, serologically and by sequence analysis. Within the project isolates of various pathogens were collected, including viruses belonging to the genus *Nepovirus s.l.* (including *Sadwavirus* and *Cheravirus*). Generally these viruses are nematodes-transmitted (*Xiphinema* spp. / *Longidorus* spp.) and can be seed transmitted to various extends. Many species have an economic impact and are regulated for European countries (under EU Directive 2000/29/EC). Research focuses on *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRSV, *Sadwavirus*), *Tomato black ring virus* (TBRV), *Tomato ringspot virus* (ToRSV) and *Tobacco ringspot virus* (TRSV). Over 50 isolates were characterized using indicator-plant research, ELISA and sequencing of important genome fragments (proteinase-polymerase gene region, polymerase and coat protein genes). Experimental data on the various nepovirus species and techniques, will be presented. The characteristics of the virus isolates are publicly accessible via Q-bank databases for regulated plant pests (www.q-bank.eu), an initiative of the Dutch Ministry of Agriculture, Nature and Food Quality. Physical collections are maintained, and can be obtained via the website.

Host and life strategy adaptations mediate competition among isolates of *Aspergillus flavus*
H. L. MEHL (1), P. J. Cotty (1)
(1) USDA-ARS, Tucson, AZ, U.S.A.
Phytopathology 100:S81

Communities of *Aspergillus flavus*, the primary causal agent of aflatoxin contamination in crops, are composed of diverse isolates that collectively cause aflatoxin contamination. Isolates vary in competitive ability on maize, but it is unclear the extent to which host-specific interactions determine success of individual isolates during competition. Seed from maize, cotton, sorghum, and soybean were surface-sterilized (80°C, 45 sec) and coinoculated with pairs of isolates previously identified as the most and least competitive on maize kernels. Czapek's agar was seeded with the same isolate mixtures. After 7 days at 31°C, isolate-specific SNPs were quantified by pyrosequencing. DNA from mycelia was used to compare colonizing abilities and DNA from conidia was used to quantify sporulation during competition. Isolates most competitive on maize were not always most competitive on other hosts. Relative colonizing ability was equal to relative ability to sporulate when isolates were grown on Czapek's agar. However, during competition on plant hosts, some isolates were better colonizers than sporulators and the extent to which an isolate competed during either colonization or sporulation varied by host. In general, less competitive colonizers were more competitive as sporulators, suggesting adaptation to different life strategies. Host and life strategy adaptations may modulate diversity within *A. flavus* communities.

Sensitive detection and discrimination of different *Pepino mosaic virus* genotypes
N. MEHLE (1), I. Gutierrez-Aguirre (1), N. Prezelj (1), D. Delic (2), U. Vidic (1), P. Kramberger (3), M. Ravnikar (1)
(1) National Institute of Biology, Ljubljana, SLOVENIA; (2) University of Banjaluka, Faculty of Agriculture, Banjaluka, BOSNIA-HERZEGOVINA; (3) BIA Separations d.o.o, Ljubljana, SLOVENIA
Phytopathology 100:S81

We developed quantitative real time PCR (RT-qPCR) assays for the detection and discremination of presently circulating PepMV (*Pepino mosaic virus)* genotypes. The following genotype combinations, European tomato-Peruvian, Ch2, and US1, were successfully distinguished within the tested isolates. One-step RT-qPCR detected PepMV European tomato genotype particles at least two orders of magnitude more sensitively than ELISA. The method detected as little as one naturally infected seed among 5000 uninfected seeds. The developed RT-qPCR assay will enable detailed studies on the demographic distribution of the circulating PepMV genotypes. At the same time, the disposability of more than one gene target for the detection of PepMV RNA, will increase the reliability of the virus detection in samples with low expected virus concentration, such as latent infections or irrigation waters. It is suspected that PepMV can be present in the environment at concentrations, which despite being too low to be detected by conventional detection methods, they can still constitute a risk of potential infection. We have experimentally confirmed that PepMV remained infective in water for up to three weeks. We are investigating the potential of monolithic chromatographic supports (CIM Convective Interaction Media) for concentration of PepMV. The combination of both technologies (CIM for concentration and qPCR for detection) will allow detection of extremely low concentrations of PepMV from environmental waters.

Fruit flesh type and harvest method affect postharvest decay of southern highbush blueberry
L. MEHRA (1), H. Scherm (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S81

Postharvest decay is a major problem in all blueberry growing areas of the U.S. Since the risk of infection is increased by fruit bruising, which in turn is increased by machine-harvest, it has not been possible to harvest southern highbush blueberry (SHB) fruit mechanically for the fresh-market. This may change with the advent of SHB genotypes with crispy berries, i.e., fruit with qualitatively firmer flesh and/or skin. Four SHB genotypes having crispy berries and four with conventional berries were either hand-picked or machine-harvested in April 2009. Fruit were sorted, packed, and placed in cold-storage (2°C) for 0, 7, 14, or 21 days. Counts of total aerobic bacteria, yeasts and molds, coliforms, and E. coli on fruit samples from the 0-day storage period were below commercial tolerance levels. Natural decay incidence following cold-storage was lowest for hand-harvested crispy fruit and highest for machine-harvested conventional fruit. Interestingly, machine-harvested crispy fruit had the same decay incidence as hand-picked conventional fruit. Across all treatments, fruit firmness was a good indicator of natural decay incidence. In a separate experiment, samples from the 0-day storage period were inoculated with *Alternaria*, *Botrytis*, and *Colletotrichum* spp, and fruit decay was assessed after 7 days in the cold room. Artificial inoculation caused less decay on crispy and hand-harvested berries, but the magnitude and significance level varied by pathogen species.

Genetically diverse isolates of *Grapevine virus A* are present in Washington vineyards
T. MEKURIA (1), R. A. Naidu (2)
(1) Washington State University, IAREC, Prosser, WA, U.S.A.; (2) Washington State University, Irrigated Agriculture Research and Exten Ctr, Prosser, WA, U.S.A.
Phytopathology 100:S81

Grapevine virus A (GVA; genus *Vitivirus*, family *Flexiviridae*) is the putative agent of grapevine kober stem grooving disorder of the Rugose Wood complex. The virus has a worldwide distribution and known to be transmitted to grapevine by some species of mealybugs and soft scale insects. Since kober stem grooving symptoms usually develop in grapevines grafted onto a rootstock but remain latent in ungrafted vines, the presence of GVA was investigated in own-rooted grapevines in Washington vineyards. Petiole extracts from six wine grape cultivars (Cabernet Sauvignon, Chardonnay, Lemberger, Merlot, Pinot Noir and Zinfandel) were tested by one step-single tube reverse transcription (RT)-PCR using primers specific to the coat protein (CP) and the RNA-dependent RNA polymerase (RdRp) domain of the replicase gene. The amplicons were cloned in pCR2.1 plasmid vector and the

sequence of gene-specific clones determined in both orientations. Pairwise comparison of the CP nucleotide sequences showed 77 to 100% identity between Washington isolates and 75 to 93% identities with corresponding sequences available in GenBank. Pairwise comparison of RdRp nucleotide sequences showed 74 to 100% identity between Washington isolates and 74 to 87% identity with other isolates from GenBank. These results indicated the presence of genetically diverse isolates of GVA in Washington vineyards. Additional data will be presented on phylogenetic relationships of GVA isolates.

The relationship between endophytic *Bacillus cereus* and *Bacillus cereus* causing foodborne illness

R. MELNICK (1)
(1) Penn State University, University Park, PA, U.S.A.
Phytopathology 100:S82

Bacillus cereus is a common inhabitant of soil as well as the endophytic portions of plant tissue. Although common in the environment, some isolates cause diarrheal foodborne illness through the production of enterotoxins. A collection of endophytic *B. cereus* isolates were obtained from several crops; including apple, cacao, tomato, and potato. PCR was conducted using 11 primer sets to amplify the genes for *B. cereus* enterotoxin T and the complexes that encode for operons of hemolysin BL (HBL) and the nonhemolytic enterotoxin (NHE). Of the 35 endophytic *B. cereus* isolates tested, the vast majority had one or more of the genes required for enterotoxin production. Approximately 14% of isolates tested lacked any enterotoxin genes. The endophytic host had no impact on the presence of enterotoxin genes, as they were detected in isolates from all tested plants. The enterotoxin genes from endophytic isolates were sequenced and compared to *B. cereus* isolates known to cause foodborne illness. Data will be presented on the presence of enterotoxin genes in environmental isolates of *B. cereus*, comparisons of sequence data between environmental and clinical samples, and whether endophytic isolates of *B. cereus* express the enterotoxin genes under differing environmental conditions.

Preliminary characterization of Tomato yellow leaf curl virus in Hawaii

M. J. Melzer (1), D. Y. Ogata (1), S. K. Fukuda (1), R. Shimabuku (2), W. B. Borth (1), D. M. Sether (1), J. S. HU (3)
(1) University of Hawaii at Manoa, Honolulu, HI, U.S.A.; (2) University of Hawaii at Manoa, Maui, HI, U.S.A.; (3) University of Hawaii, Honolulu, HI, U.S.A.
Phytopathology 100:S82

Tomato yellow leaf curl disease, caused by the begomovirusTomato yellow leaf curl virus (TYLCV; family Geminiviridae), is a destructive disease of tomato (Solanum lycopersicum L.), particularly in tropical and sub-tropical regions. In the fall of 2009, tomato plants showing stunted new growth, interveinal chlorosis, and upward curling of leaf margins were reported in Wailuku, Maui, and in Poamoho, Oahu. A ~1.5 kbp PCR product was amplified from symptomatic tomato plants using degenerate geminivirus primers, but not healthy controls. Sequence analysis revealed these products had a 92–99% nucleotide identity to TYLCV sequences in GenBank. The Wailuku isolate (GenBank accession # GU322424) appears to be more closely related to TYLCV isolates from Japan and China, whereas the Poamoho isolate (GU322423) appears to be more closely related to TYLCV isolates from the mainland U.S.A. and Mexico, suggesting the virus has been introduced into Hawaii on multiple occasions. The identification of reservoir hosts of TYLCV in Hawaii's flora is critical for disease management. Several common agricultural weed species from the Poamoho site were evaluated for the presence of TYLCV using a squash-blot hybridization assay with a digoxigenin-labeled probe derived from the ~1.5 kbp PCR amplicon. Cheeseweed (Malva parviflora L.), a reported host of TYLCV, tested positive for TYLCV using this assay as did the solanaceous weed, Apple of Peru (Nicandra physalodes L.).

Identification of *Streptomyces stelliscabiei* causing potato common scab in Michigan

Q. MENG (1), J. Hao (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S82

Four *Streptomyces* isolates were obtained from potato (*Solanum tuberosum*) tubers with common scab symptoms from a field in Michigan in 2009. Isolates were purified by single colony transfer. Genomic DNA was extracted from the isolates. Result of polymerase chain reaction (PCR), using species-specific primers, for all the isolates was positive for *Streptomyces stelliscabiei*, but negative for any of the other known pathogenic *Streptomyces* spp. 16S ribosomal RNA from the isolates was sequenced, and analyzed using the BLAST algorhythm against the NCBI Genbank. The results showed 99 (2 isolates) to 100% (2 isolates) identity to *S. stelliscabiei* depending on the isolate. The isolates were all confirmed by PCR to have *txtAB*, *nec1*, and *tomA* genes, which are associated with pathogenicity of scab-causing *Streptomyces* spp. Thaxtomin production was detected when the isolates were cultured in oat broth media. Culture plugs of the isolates were placed on sterile potato tuber slices, and necrotic lesion was observed after 3 days of incubation. Radish seeds were grown in water agar or soil, infested with one of the isolates. After one week, radish roots of plants inoculated with bacteria were darkened or showed suppressed growth. The pathogen was re-isolated from the lesion from radish roots and confirmed as identical to the original inoculum by using PCR and morphological characterization. This is the first report of *S. stelliscabiei* in Michigan.

Visual and quantitative characterization of ironwood tree (*Casuarina equisetifolia*) decline on Guam

Z. MERSHA (1), R. L. Schlub (1), P. O. Spaine (2), J. A. Smith (3), S. C. Nelson (4)
(1) UOG Cooperative Extension, University of Guam, Mangilao, GUAM; (2) USDA Forest Service, Athens, GA, U.S.A.; (3) University of Florida's Institute of Food and Agricultural Sciences, Gainesville, FL, U.S.A.; (4) Cooperative Extension Service, University of Hawaii At Manoa, Hilo, HI, U.S.A.
Phytopathology 100:S82

To assess the level of ironwood tree decline on Guam, photographs of 44 randomly selected trees with varying levels of decline were categorized into small (CBH ≤ 100 cm) or large (CBH >100 cm) based on their circumference at breast height (CBH) and visually catalogued into a five-scale decline severity (DS) rating. On subsequent surveys, trees with different DS ratings were characterized visually for branch thinning and quantitatively for branchlet ("needle") biomass. As DS increased from 0 (healthy) to 4 (nearly dead) branch thinning, progressively increased from 0 to 95.0% and 0 to 92.5% for small and large trees, respectively. There was no significant difference between branchlet biomass for DS 0 and DS 1 nor between DS 2 and DS 3 trees. The greatest branchlet weight loss, at 95.3%, occurred to DS 4 trees. Internal symptoms included various patterns of discolorations in trunks and a white soft-rot in roots. Discoloration was consistently traced into branches through cross-sectioning at the branch trunk interface. In branches the presence of discoloration was only 100% reliable for DS 3 and 4 trees. External symptoms starts at the top of trees and progresses downward; whereas, internal discoloration starts at the tree's base and diminishes acropetally This gradient of discoloration was well described by the exponential decay function ($P = 0.0001$) with R^2 values of 0.85 and 0.73 for small and large trees, respectively. Ironwood tree decline has expanded into areas such as Cocos Island, which were virtually decline free a year earlier.

Enhancing Guam's agriculture professionals' knowledge of ecological disease management

Z. MERSHA (1), R. W. Brown (1), R. L. Schlub (1)
(1) UOG Cooperative Extension, University of Guam, Mangilao, GUAM
Phytopathology 100:S82

In 2009, a plot was set up at the Yigo Experiment Station to collect data for an agriculture professional training manual on detection of soil nutrient deficiencies and the role of soil nutrients in plant health and disease suppression. Crops included tomato (*Solanum lycopersicum* cv. Season Red), eggplant (*Solanum melongena* cv. Pingtung Long), and cucumber (*Cucumis sativus* cv. Joy). Nutrient treatments were NPK, NPK plus micronutrients, deficient N plus PK, deficient P plus N K, and deficient K plus NP. Deficiency levels were at 25 percent of the current UOG Cooperative Extension recommendation. Data collected included information on diseases, sugar content of fruits, soil pH, salinity, soil nutrients content, and plant biomass, height, yield, leaf count, tissue nutrient content, and chlorophyll levels. A variety of equipments was used for data collection and for training evaluation purposes: soil salinity meter, pH meter, YSI9500 photometer nutrient analyzer, brix meter, SPAD502 chlorophyll meter, and Cardy meters (NO_3^- and K^+). Partial analysis of the data indicates that a 75% reduction in recommended amounts of N, P, or K was sufficient to impact yield but not the development of diseases or visible distinct symptoms. Cardy meters, YSI9500 photometer, and SPAD502 chlorophyll meter emerged as potential tools that can be used to detect nutrient deficiencies in the field.

Seasonal dynamics of black leaf mold (*Pseudocercospora fuligena*) on tomato (*Solanum lycopersicum*) grown under protected cultivation in Thailand

Z. MERSHA (1), B. Hau (2)
(1) University of Florida, Tropical Research and Education Centre, Homestead, FL, U.S.A.; (2) Leibniz Universitaet Hannover, Hannover, GERMANY
Phytopathology 100:S82

Epidemics of black leaf mold (BLM), caused by *Psudocercospora fuligena*, were studied in 24 plantings of tomato (*Solanum lycopersicum* cv. FMTT260)

under naturally ventilated greenhouse condition in central Thailand from May 2005 to March 2007. Three BLM peak-epidemics periods were identified: August-September in 2005 (I) and 2006 (II), and plantings in December 2005-January 2006 (III). Mean BLM severity (*DS**) of 0.30, 0.14 and 0.20 were recorded from I, II and III, respectively. Favorability indices of temperature (FI_T) and relative humidity (FI_{RH}) of the respective peak periods were 0.90, 0.61 and 0.66 for FI_T and 0.51, 0.35 and 0.44 for FI_{RH}. Linear, multiple and stepwise regressions were performed to detect weather-disease-host relationships. FI_{RH} was highly correlated with *DS** ($r^2 = 0.71$) and the multiple regression of FI_{RH} and FI_T in the model $DS^*=a+(b^*FI_T)+(d^*FI_T*FI_{RH})$ improved R^2 value to 0.74 with significant parameter estimates. Maximum plant height (*MPH*) of all experimental plants was fairly correlated ($r^2 = 0.32$, $p < 0.0001$) with marketable yield (*MY*). Despite the poor linear correlation between *MY* and *DS**, integration of *MPH* in the model $MY = (a+b^*MPH)^*(1-c^*DS^*)$ resulted in R^2 value of 0.35 and high significance of the parameters *b* and *c*. A 1% *DS**, according to this model, reduced *MY* by ≅ 1.2%. The actual yield losses recorded from comparison of sprayed and non-sprayed treatments of I, II and III were 34.1, 39.2 and 20.6%, respectively. The last model explained the actual yield losses of I and III but the loss in II was higher than the prediction.

Efficacy of acibenzolar-S-methyl and copper fungicides for the control of angular leaf spot of strawberry

J. C. MERTELY (1)
(1) University of Florida-GCREC, Wimauma, FL, U.S.A.
Phytopathology 100:S83

Angular leaf spot (ALS), caused by *Xanthomonas fragariae*, is an occasionally severe disease of strawberry The most common symptoms are spotting and blighting of the leaves, although the calyx may also be infected, rendering the fruit unsightly and unmarketable. Products for controlling ALS have been tested in replicated field trials at the Gulf Coast Research and Education Center in Wimauma, Florida since 2005–06. Test products are typically applied as foliar sprays on a 7-day calendar schedule throughout the winter growing season. Over the past five seasons, representative biopesticides, copper fungicides, and organic fungicides have been tested, as well as acibenzolar-S-methyl, hydrogen peroxide, and kasugamycin. To date, acibenzolar-S-methyl applied at 26 g a.i./ha and copper fungicides applied at ca. 300 g metallic copper/ha have given the greatest and most consistent disease suppression. Higher rates often improved disease suppression, but did not increase yield due to copper phytotoxicity or the metabolic costs associated with acibenzolar-S-methyl. During the 2008–09 season, when up to 40% of the foliage was killed or damaged by ALS, weekly applications of acibenzolar-S-methyl at 26 g a.i./ha or copper hydroxide at 294 g metallic copper/ha significantly increased marketable yield. Yield improvements were not demonstrated under conditions of lower disease pressure in the other four trials.

Pomegranate decay caused by *Pilidiella granati* in California

T. J. MICHAILIDES (1), R. Puckett (1), D. Morgan (1)
(1) University of California Davis, Parlier, CA, U.S.A.
Phytopathology 100:S83

During the last decade and after the recent discoveries of the high antioxidant content of pomegranate fruit and juice, the pomegranate industry has increased to >20,000 acres in California. Pomegranates are marketed as intact fresh fruit, extracted arils, or juice. Fruit diseases such as black heart caused by *Alternaria* spp., Aspergillus and Penicillium rots, are considered the most important diseases of pomegranate fruit. However, in the last two years, another rot caused by a pycnidial fungus *Pilidiella granati* has been commonly observed in fruit from fields or packinghouses. Pilidiella rot is very different from black heart rot in that it decays the arils and the fruit rind while black heart decays only the arils. On APDA, *P. granati* produces white to light yellow, leathery mycelia with abundant black, solitary pycnidia of various sizes. Optimum temperature of growth on APDA ranges from 25 to 30°C; the fungus grows at 15°C, but not at 35°C. Although infection needs a wound, infection moved from infected fruit to intact fruits in contact. The pathogen overwinters as pycnidia and mycelia in rotten fruit in the field. It has been isolated also from dying shoots of young pomegranate trees. *P. granati* is characterized by the hyaline to pale brown conidia (length:width >1.5) in contrast to *Coniella* spp. which have dark brown conidia (length:width <1.5). To our knowledge, this is the first report of *P. granati* causing significant fruit rots of pomegranate in California.

Anthracnose fruit rot resistance in blueberries: Evaluation of a rapid screening technique and correlation with fruit characteristics

T. D. MILES (1), J. F. Hancock (1), P. W. Callow (1), A. M. C. Schilder (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S83

Anthracnose fruit rot, caused by the fungus *Colletotrichum acutatum*, is an important disease of blueberries. The objectives of this study were to evaluate a rapid screening technique for anthracnose fruit rot resistance and to determine if certain fruit characteristics are correlated with resistance. Cut surfaces of ripe fruit were inoculated with a *C. acutatum* suspension (10^6 conidia/ mL) in two replicated experiments with 24 and 26 blueberry cultivars, respectively. Resistance was evaluated by quantifying sporulation on the cut fruit surface after 3 days of incubation at 22–24°C. Resistance in this bioassay correlated well with published resistance ratings (Polashock *et al.* 2005). Fruit pH, titratable acids, sugar content, and firmness of the cultivars were assessed from 2005 to 2008. A negative linear correlation was found between fruit rot incidence and sugar content ($P = 0.001$, $R^2 = 0.36$). The direct effect of sugar concentration (1%, 2%, 4%, 8%, and 16% glucose or fructose) and pH (2.0, 2.5, 3.0, 3.5, 4.0, 5.5, 7.0) on hyphal growth of *C. acutatum* on defined media was evaluated. A reduction in hyphal growth was observed at pH values below 3.5 and sugar concentrations above 8%. The roles of pH and sugar concentration in fruit rot resistance require further investigation. The cut-fruit inoculation method provides a rapid means for fruit rot resistance screening in blueberries and requires less fruit than conventional whole fruit inoculation methods.

Identifying anthracnose disease resistant strawberry clones using traditional techniques and molecular markers for screening

M. MILLER-BUTLER (1), B. Krieser (1), K. J. Curry (1), B. J. Smith (2)
(1) University of Southern Mississippi, Department of Biological Sciences, Hattiesburg, MS, U.S.A.; (2) USDA ARS Southern Horticultural Laboratory, Small Fruit Research Unit, Poplarville, MS, U.S.A.
Phytopathology 100:S83

Anthracnose of commercially grown strawberry, *Fragaria* × *ananassa*, may be caused by any of three *Colletotrichum* species: *C. acutatum*, *C. fragariae*, or *C. gloeosporioides*. These destructive pathogens may cause fruit rot, leaf spot, petiole lesions, crown rot, wilt, and death of the plant. Traditional and molecular approaches were used to identify anthracnose resistant strawberry clones. We used a traditional disease screening method, i.e., inoculating detached leaves of 52 strawberry clones with conidial suspensions of two *C. fragariae* isolates and one *C. gloeosporioides* isolate and determined that 48% were susceptible, 23% were intermediate, and 29% were resistant to anthracnose based on their disease score. Researchers have identified two molecular markers linked to the *Rca2* gene found in strawberry germplasm resistant to *C. acutatum*. These two markers were used to screen the 52 strawberry clones for the presence/absence of this gene. Thirty-seven percent of the clones had both markers, 50% had one of the markers, and 4% had neither marker. More strawberry clones will be screened for the presence of these two markers and for anthracnose resistance using traditional techniques. Correlation of the presence or absence of the gene markers with resistance or susceptibility to anthracnose caused by any of the three *Colletotrichum* species should decrease the time it takes to screen selections for anthracnose-resistant genotypes in strawberry breeding programs.

Occurance of *Prothallonema asymmetricum* Siddiqi, 1986 in Arak Province of Iran

M. MIRZAEE-QOMI (1), F. Khozeini (2), S. Barooti (3), S. Rezaee (1)
(1) Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Plant Protection Ministry, Iranian Research Institute of Plant Protection, Tehran, IRAN; (3) Nematology Research Department, Iranian Research Institute of Plant Protection, Tehran, IRAN
Phytopathology 100:S83

During a survey in Arak county of Iran a species belonging to the genus Prothallonema was isolated. Based on morphological and morphometrics studies, it was indentified as *Styctylus asymmetricus* Thorne,1941 with new scientific name as *Prothallonema asymmetricum* siddiqi,1986. Iranian specimen extracted from alfalfa soil of Marzijaran region in Arak porovince of Iran. Females contained a thin layer of culticul over their head with a three partite 10 µm stylet with asymmetric knobs. Dorsal knobs of spear much smaller than the others crops of esophagus fusiform. Basal bulb esophagus ovoid to pyriform with elongated stem extending into intestine. A morphometric characteristic feature of this nematode is esophagus in which the terminal bulb was envaginated with intestine. Ovary outstretched with one or two flexures. Posterior uterine branch absent. Nematodes contained 4 laterals in which the width was quarter to third of the body width. Esophagal duct was located on the end of the stylet and D.G.O was exactly located on the beneath of the knobs. Females were Mono-Pro- Delphic and vulva was near to the annus. In against of young nematodes with long mono-prodelphic ovary, older contains a amphi-delphic ovary with one or two inverts. V% was also about 90% in females. Except for sexual organs all other parts of male bodies were same as what has observed in females. Compared to females with 1000 µm length, males were shorter. This is the first report of this nematode in Iran.

The powder formulation survey of strain *Bacillus subtilis* M36 for control of bean root rot caused by *Fusarium solani* f. sp. *phaseoli*

P. MIRZAEE-QOMI (1), H. Zamanizadeh (1), R. Saberi-Riseh (2), M. Lak (3)
(1) Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Department of Plant Pathology & Entomology, College of Agriculture, University of Tehran, Rafsanjan, IRAN; (3) Central Province Agricultural Research and Natural Resources Center, Arak, IRAN
Phytopathology 100:S84

In the survey 194 gram posetive bacteria isolated from the rhizosphere of bean from Markazi province and also 41 isolates were prepared from the collection of Research Center of Biological Control at Tehran Universitiy were evaluated on the basis of inhibitionry zone of fungal growth. Finaly eight the better strain of *B. subtilis* were selected for greenhouse trials. Result showed that antagonistic bacterium B. s M36, isolated from the rhizosphere of bean fields Markazi province exhibited the greatest effect on reducing the Fusarium root rot bean. In greenhouse trials, after six weeks, between nine formulations of strain B. s M36, the one which obtained from M1 culture medium with Arabic Gum as the sticker with 68.42% control, depicts the greatest effect on reducing Fusarium root rot was more effective than two fungicides Rovral-TS and Trichomix-HV with 56.14% and 54.38% control respectively. The result showed that the population of B. s M36 grown from in powder formulations has decreased during the storage at 4 and 25°C over a 150-day period. In addition, formulation of strain B. s M36 stored at 4°C had longer shelf life than those stored at 25°C. Long-term stability of formulation (M1-AG) of strain B. s M36 was the best. Study of media on population of effective strain, exhibited that the M1 medium had the most effect in this study.

Addressing the relationship between *Pseudoperonospora cubensis* and *P. humuli* using multigenic and host specificity assays

M. N. MITCHELL (1), D. H. Gent (2)
(1) Oregon State University, Corvallis, OR, U.S.A.; (2) USDA ARS NFSPRC/ OSU BPP, Corvallis, OR, U.S.A.
Phytopathology 100:S84

The pathogens *Pseudoperonospora cubensis* and *P. humuli*, the causal agents of the downy mildews on cucurbits and hops respectively, have been demonstrated to be sister taxa. Species concepts in downy mildews are in flux and include morphology, host range, reproductive isolation and molecular evidence. A recent study that examined sequence data from the internal transcribed spacer (ITS) and morphological characteristics of both pathogens suggested that the species are synonymous. As nomenclature has implications for pathogen identification, disease management tactics, and plant quarantine regulations, it would be significant to resolve whether the synonymy of *P. cubensis* and *P. humuli* is correct. To increase resolution, the mitochondrial cytochrome oxidase (*cox*) gene cluster, and two nuclear loci, ITS and β-tubulin, were analyzed with parsimony and Bayesian approaches. For nuclear loci, all but two isolates of *P. humuli* were moderately to strongly resolved as a clade within *P. cubensis*. However, the *cox* cluster showed the opposite relationship. Host specificity experiments performed on universally susceptible cucurbit hosts and hop cultivars indicate that the pathogens have discrete pathogenic capabilities. Thus, there is evidence that there exist biologically relevant characteristics that differentiate the two organisms with implications for the detection and management of both that may be concealed by the reduction of *P. humuli* to a taxonomic synonym of *P. cubensis*.

Cercospora and Corynespora leaf spots in *Hydrangea macrophylla*

M. T. MMBAGA (1)
(1) Tennessee State University School of Agriculture and Consumer Sciences, McMinnville, TN, U.S.A.
Phytopathology 100:S84

Leaf spots and leaf blight symptoms observed in garden hydrangea in mid-Tennessee were identical to those described for both Cercospora and Corynespora leaf spot. Pathogenicity tests with isolated organisms indicated that the leaf spots/ blight symptom was caused by several pathogens. Spores of *Corynespora cassiicola* and *Cercospora hydrangeae* were observed on leaf samples that showed identical symptoms. In some samples, spores of both *C. cassiicola* and *C. hydrangeae* were observed in the same lesions, but only *C. cassiicola* was isolated in culture. On other samples, lesions were infected with only *C. cassiicola* or *C. hydrangeae*. Single spore isolation from leaf lesions resulted in pure cultures of both *C. cassiicola* and *C. hydrangeae*. These fungi did not sporulate well when cultured on Potato Dextrose Agar. Morphological characteristics and DNA sequence analysis were used to identify the pathogens and specific primers for *C. cassiicola* and *C. hydrangeae* were designed to identify the two pathogens on infected leaves. Differences in the virulence and aggressiveness of *C. cassiicola* and *C. hydrangeae* on 77 hydrangea cultivars showed that *C. cassiicola* is a highly aggressive pathogen and demonstrated the role of each pathogen in the leaf spot/blight disease complex.

Leaf spot disease in ornamental flowering cherry nursery production

M. T. MMBAGA (1), L. Mackasmiel (2), J. Joshua (3)
(1) Tennessee State University School of Agriculture and Consumer Sciences, McMinnville, TN, U.S.A.; (2) Tennessee State University, McMinnville, TN, U.S.A.; (3) Tennessee State University, Nashville, TN, U.S.A.
Phytopathology 100:S84

Ornamental flowering cherry trees are grown primarily for their floral display in early spring, but they are closely related to fruit cherry trees that are grown for their fruits. A leaf spot disease emerged recently in a Tennessee nursery causing great concern to growers. The disease is characterized by small red-brown spots and shot holes that often merge to form large irregular lesions and shot holes. Fungicide applications were initiated soon after disease symptoms were observed, but the grower had no guidance on effective fungicides or the timing of spray program and disease control failed. The disease caused severe defoliation; by June, only a few leaves remained on infected trees. Persistent wet weather throughout 2009 favored disease development and plant vigor was significantly reduced. Infected trees had severe winter injury and reduced market value. Disease symptoms were consistent with that of cherry leaf spot, a well known problem in stone fruit trees. Source of infection was traced back to the liners and cuttings. Although some cultivars appeared to be resistant to the disease, the resistance has not been confirmed. The integration of copper based fungicide (Captan), with a Demethylation inhibitor (Tebuconazole) and a QoI fungicide (Trifloxystrobin) have been effective in fruit trees and were evaluated on ornamental cherry following a spray program based on local weather.

A chemo-thermotherapy technique for eliminating viruses from rose plants

A. MODARRESI CHAHARDEHI (1), J. Mozafari (2), F. Rakhshandehroo (3), D. Ibrahim (1)
(1) School of Biological Sciences, Universiti Sains Malaysia (USM), Penang, MALAYSIA; (2) Department of Genetics and National Plant Gene-Bank, Seed and Plant Improvement Institute, Karaj, IRAN; (3) Department of Plant Pathology, Islamic Azad University, Science and Research Branch, Tehran, IRAN
Phytopathology 100:S84

Arabis mosaic virus (ArMV) and *Prunus necrotic ringspot virus* (PNRSV) are among the most commonly found viral agents on roses. Conventional method of thermotherapy is not an efficient technique in regenerating virus-free plants. Using a combination of thermotherapy, chemotherapy and *in vitro* culture we have developed a procedure for eliminating ArMV and PNRSV from rose plants. Viral infected rose stem nodal segments were surface sterilized and cultured on MS media containing 0.4 mg/l NAA, 0.4 mg/l BAP (pH 5.8) and 10, 20 or 30 mg/ml of ribavirin. After 20 to 40 days, regenerated segments were excised and transferred to new media and were tested for virus titer by enzyme linked immune-sorbent assay (ELISA). In addition to ribavirin treatment plants were also subjected to combination of ribavirin and thermotherapy treatments where ribavirin treated plants after 30 days were exposed to the temperature of 38°C for 16 hours and 22°C for 8 hours. Results of chemotherapy showed that complex of PNRSV and ArMV was efficiently eradicated from plantlets that were grown on MS medium containing 10 and 30 mg/l for 40 and 20 days respectively. The virus elimination rates of 63.33 and 85.18% were obtained for ArMV and ArMV+PNRSV, respectively. However, thermotherapy along with chemotherapy (containing 30 mg/l) during the period of four weeks was the most effective treatment for plantlet regeneration and virus elimination. Explant cultures with ribavirin appears to be a simple and effective way for obtaining virus-free roses *in vitro*.

Effect of resistant catch crops on population decline of sugar beet cyst nematode

A. Mohammad Deimi (1), S. BAROUTI (1)
(1) Dept. of Plant Pathology, Faculty of Agriculture and Natural Resources, Islamic Azad University, Tehran, IRAN
Phytopathology 100:S84

The use of resistant catch crops is an important element of crop rotation in integrated nematode management. Therefore, during the 2009, experiments were undertaken to determine the effects of some cultivars of oil radish (*Raphanus sativus*), white Mustard (*Sinapis alba*), Buckwheat (*Fagopyrum esculentum*) and *Phacelia tanacetifolia* on population decline of sugar beet cyst nematode (sben). Plants were grown after the harvest of winterwheat and were collected 130,100 and 78 days later respectively. Ratio between final populations (pf) to initial population (pi) of the nematode was 60% for *S. alba* Cv. Maxi and *R. sativus* Cv's Nemex and pegletta and the difference was significant at the 1% level comparing to the untreated controls.

Characterization of plant cell wall-degrading enzymes produced by *Pantoea stewartii* subsp. *stewartii*, the causal agent of Stewart's wilt of corn

M. MOHAMMADI (1), L. Burbank (1), C. M. Roper (1)
(1) University of California, Riverside, CA, U.S.A.
Phytopathology 100:S85

Mojtaba Mohammadi, Lindsey Burbank and Caroline Roper *Pantoea stewartii* subsp. *stewartii* (*Pnss*), a xylem-dwelling bacterium, is the causal agent of Stewart's wilt and blight of sweet corn and maize. The goal of this study is to investigate if *Pnss* produces extracellular enzymes and to determine their contribution to virulence on corn. Carboxymethyl cellulase (CMCase), β-1,4-endoglucanase, β-1,4-xylanase and mixed-linkage β 1,3-1,4 glucanase activities were detected and measured in sonicated cells and culture supernatants of *Pnss* (DC283). Maximum specific activities occurred during the mid-log and early stationary phases, suggesting these enzymes are produced in a cell-density dependent manner. Electrophoresis of non-denaturing polyacrylamide gels impregnated with either β-D-glucan or carboxymethyl cellulose (CMC) revealed the presence of two isoforms of CMCase and β-1,4-D-endoglucanase whose molecular weights were in the range of 55–60 kDa. Furthermore, HPLC analysis of the reducing sugars released during the incubation of *Pnss* (DC283) culture supernants with either CMC or β-1,4 glucan revealed the presence of several oligosaccharide hydrolysis products indicative of enzymatic degradation. Additionally, work is currently under way to characterize mutants of *Pnss* DC283 lacking the β -1,4-endoglucanase and xylanase genes to elucidate their role in virulence on corn seedlings.

Effect of VAM colonization in pistachio rootstock on growth, nutrition and Phytophthora root rot

A. Mohammadi (1), Z. BANIHASHEMI (2)
(1) Iran Pistachio Research Center, Rafsenjan, IRAN; (2) Shiraz University, Shiraz, IRAN
Phytopathology 100:S85

Phytophthora root and crown rot caused by *Phytophthora* spp. is one of the major limiting factors in pistschio inflicting economic losses in Iran. Arbuscular Vesicular Mycorrhizal (VAM) fungi are the most common type of plant symbiosis in natural and agricultural ecosystems. The objective of the present experiment was to study the effect of *Glomus mosseae* colonization on growth, plant nutrition and root rot caused by *P. drechsleri* in susceptible (Sarakhs) and tolerant (Qazvini and Atlantica) pistachio rootstocks under greenhouse conditions. *G. mosseae* was inoculated to pistachio rootstocks prior to pathogen inoculation. In the absence of *P. drechsleri*, mycorrhized pistachio seedlings had higher shoot and root dry weight, height and concentration of P, K, Ca, Mg, Fe, Cu, Zn and Mn than non-mycorrhized plants. Inoculation of *P. drechsleri* in non-mycorrhized susceptible seedlings caused in significant reduction of the above mentioned parameters compared to none inoculated control, but low or no reduction was observed in tolerant rootstocks. Non-mycorrhized susceptible cultivar died 40 days after inoculation but the mycorrhized one delayed and decreased their mortality. It is concluded that mycorrhizal colonization in pistachio seedlings increased growth, nutrition and decreased mortality by *P. drechsleri* than non-mycorrhizal one especially in susceptible rootstocks.

Activity of hydrolytic enzymes and antioxidants in mycorrhized pistachio root infected by *Phytophthora drechsleri*

A. Mohammadi (1), Z. BANIHASHEMI (2)
(1) Iran Pistachio Research Center, Rafsenjan, IRAN; (2) Shiraz University, Shiraz, IRAN
Phytopathology 100:S85

The role of hydrolytic enzymes (chitinase, chitosanase, β-1,3-glucanase), antioxidants (GPX, CAT, SOD) and PAL was evaluated in *Glomus mosseae* (Gm) colonized susceptible (cv. Sarakhs) and tolerant (cvs. Qazvini and Atlantica) pistachio rootstocks in the presence of *Phytophthora drechsleri* (Pd). Increasing activity of hydrolytic enzymes coincided with the increase of Gm colonization. After establishment of mycorrhiza, the enzymatic activity was decreased but was higher than the non-mycorrhizal controls. In mycorrhized seedlings, PAL, GPX and CAT activity increased at the first stages of growth and establishment of Gm and then decreased but SOD activity was unchanged or in some cases had increasing trend. Inoculation of Pd, resulted in higher activity of antioxidants and PAL which began earlier and higher in tolerant than in susceptible rootstocks. In Gm+Pd treatments, PAL and antioxidants activity was unchanged or in some cases increased slightly and then decreased but hydrolytic enzymes activity increased in all rootstocks. In tolerant cultivars, higher and earlier activity of the enzymes was observed than susceptible one. Reduction of Pd population in the mycorrhizosphere resulted in the reduction of hydrolytic enzyme activity. These results indicated that inoculation of Gm in pistachio rootstocks prior to Pd can activate defense related enzymes and protect pistachio seedlings against root rot especially in susceptible rootstocks.

Effect of pH on the growth of *Rhizoctonia* spp. from cereal-based cropping systems in eastern Washington State

A. MOHD JAAFFAR (1), D. M. Weller (2), T. C. Paulitz (2), L. S. Thomashow (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA-Agricultural Research Service, Pullman, WA, U.S.A.
Phytopathology 100:S85

Rhizoctonia root rot caused by *Rhizoctonia solani* AG 8 and *Rhizoctonia oryzae* are serious root diseases in dryland cereal production in Washington State. Isolates of *Rhizoctonia* spp. from fields with different cropping histories in the low- (<12 inches) and moderate- (>12 inches) precipitation zones of eastern Washington State were identified by using six PCR primer sets specific for AG 8, AG 2-1, AG 10, AG I (*Ceratobasidium* spp.) and 2 groups of *R. oryzae*. Of 205 isolates, AG 8, AG I, AG 2-1, *R. oryzae* group 2, *R. oryzae* group 3, and AG 10 comprised 28%, 19%, 17%, 15%, 13%, and 8% of the isolates, respectively. AG 8 comprised 67% and 33% of the isolates in the low and moderate precipitation zones. The soil pH in fields in eastern Washington varies considerably (<pH 4 to >pH 7.5) because of decades of use of ammonium fertilizer, which lowers soil pH. *Rhizoctonia* isolates were tested for growth on 1/5-strength PDA adjusted to a range of pH values from 5.6 to 7.6. AG groups differed significantly in growth at the various pH values and in general, growth was significantly reduced above pH 6.8. These results suggest that the composition of *Rhizoctonia* isolates in a field may be determined in part by the soil pH and precipitation.

Identifying heterokaryon incompatibility loci in *Aspergillus flavus* and *Aspergillus parasiticus* using array-Comparative Genome Hybridization (aCGH)

J. T. MONACELL (1), B. W. Horn (2), R. Singh (3), E. A. Stone (4), I. Carbone (3)
(1) Department of Plant Pathology and Bioinformatics Research Center, North Carolina State University, Raleigh, NC, U.S.A.; (2) National Peanut Research Laboratory, USDA, ARS, Dawson, GA, U.S.A.; (3) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (4) Department of Genetics and Bioinformatics Research Center, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S85

Heterokaryon incompatibility is the inability of two strains to undergo fusion of vegetative fungal cells. This vegetative compatibility system is dictated by a series of heterokaryon incompatibility (het) loci whose alleles must all be identical for stable hyphal fusions to occur. Het loci have been identified in several filamentous fungi, but are currently unknown in Aspergillus flavus and A. parasiticus. These species are agriculturally important plant pathogens that produce the potent carcinogens, aflatoxins. Fungal individuals can be grouped into vegetative compatibility groups (VCGs) based on their ability to undergo hyphal fusions and potentially form heterokaryons. We performed aCGH for eleven VCGs and a total of 51 strains in Aspergillus section Flavi, including A. flavus, A. parasiticus, A. oryzae, A. caelatus, A. tamarii and A. nomius. We conducted an initial screening of these data for signatures of balancing selection around single-feature polymorphism markers on chromosomes 2, 3, 4, and 6, which associated one-to-one with VCG. Our screening for evidence of balancing selection has revealed several putative het loci showing distinct patterns of trans-speciation, which is typical of other loci under balancing selection in these fungi, such as mating-type genes and the aflatoxin gene cluster. Among the candidate het loci we identified using aCGH was a previously annotated putative het locus on chromosome 2.

Cultivar susceptibility influences the leaf wetness duration (LWD) and temperature relationship of *Alternaria alternata* citrus infection

S. N. Mondal (1), L. P. Timmer (1), M. M. DEWDNEY (1)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S85

Alternaria brown spot (ABS), caused by *A. alternata*, is an important fresh market disease of tangerines and tangerine hybrids. The Alter-Rater, a predictive model for better timing fungicide applications, was developed with the very susceptible Minneola but it was unknown whether the LWDs and temperatures needed for infection would be similar for all tangerine hybrids. Leaf wetness duration and temperature relationships were tested on 5 tangerine hybrids: Dancy, Minneola, Murcott, Nova and Sunburst. The LWDs were 2, 4, 8, 16, 24 and 30 h at temperatures of 20, 24, 28 and 32°C. The rating scale for number of lesions/leaf was: 0=0; 1=1-2; 2=3-5; 3=6-10; 4=11-15; 5=>15 lesions/leaf and the data were taken from 15 leaves/plant. Cultivar differences were observed with two susceptibility groups: highly susceptible, Minneola and Dancy, and moderately susceptible Murcott, Nova and Sunburst ($P < 0.0001$). As few as 2 h of LWD were needed for light infection but 16-24 h, severe infection occurred on all cultivars and temperatures. The optimal temperature range for lesion production was 24 and 28°C for all LWDs and the quadratic effect of temperature was highly significant ($P < 0.0001$). The

interactions of temperature and cultivar as well as temperature and LWD were highly significant ($P < 0.0001$) but LWD and cultivar was not. Results will be incorporated into the Alter-Rater model so that less susceptible tangerine and tangerine hybrids are not sprayed unnecessarily.

Evaluation of transgenic grapefruit (*Citrus paradisi*) for resistance to citrus scab caused by *Elsinoë fawcetti*

S. N. Mondal (1), M. Dutt (1), G. A. Barthe (1), J. W. Grosser (1), M. M. DEWDNEY (1)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S86

Citrus scab, *E. fawcettii* (anamorph *Sphaceloma fawcettii*), is a common foliar fungal disease, occurring in most humid citrus growing areas of the world. The scab pathogen infects juvenile leaves, twigs and fruit of most citrus species, including grapefruit, and can result in severely blemished fresh fruit. Since no grapefruit cultivar is resistant to scab, transgenic plants offer potential resistance to this important disease. In the present study, several transgenic grapefruit lines containing a synthetic cecropin A-melittin chimeric lytic peptide gene (LIMA) under control of a constitutive d35S promoter were evaluated for resistance to *E. fawcettii*. Detached leaf assay results with the Russel-15 isolate (Florida broad host range pathotype) indicated that several lines produced significantly fewer raised pustules when compared to the control ($P < 0.05$). Similar results were recorded in greenhouse assays where 6 transgenic lines produced significantly less scab compared to control ($P < 0.05$). In sporulation assays, repeated measures ANOVA revealed significant variation in the effect of transgenic lines on spore production over time. All transgenic lines produced significantly fewer conidia in all blocks. Pustules of untransformed grapefruit leaves increased conidia production unlike most transgenic lines evaluated. Northern blots indicated varying levels of gene expression while PCR and Southern blots confirmed stable integration of the gene in the plant genome.

Identification and characterization of genes involved in the type VI secretion system in *Xanthomonas axonopodis* pv. *manihotis*

N. MONTENEGRO (1), A. Alvarez (1), S. Restrepo (1), A. J. Bernal (1)
(1) Universidad de Los Andes, Bogotá, COLOMBIA
Phytopathology 100:S86

The type VI protein secretion system (T6SS) in gram-negative bacteria has been shown to participate in several cellular processes, including pathogenesis. During pathogenesis of *Pseudomonas aeruginosa* and *Vibrio cholerae*, this system secretes effector proteins that interact with target proteins in the host cell, thus contributing to the infection process. The system has been studied in animal pathogens and some substrates have been identified with roles in a diverse set of processes. However, there is a paucity of data for the importance of this system in plant pathogenic bacteria-host interactions. In this study, we have identified the presence of the T6SS in *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), the causal agent of bacterial blight in cassava (*Manihot esculenta*). Bioinformatics analysis of nine structural genes (e.g. *clpB*, *vgrG*, *ompA*, *hcp* and *fha*) and six candidate genes, confirmed the existence of a full T6SS in *Xam*. We subsequently used site-directed mutagenesis of some of the structural genes involved in the T6SS. These mutants were inoculated in susceptible plants and the results suggest an importance of this secretion system in the pathogenicity of *Xam*. This study shows the importance of the novel T6SS in the virulence of Xanthomonas and it is considerably relevant for the understanding of plant- pathogen interactions.

Brief gaseous shocks to inhibit postharvest gray mold on 'Mollar de Elche' pomegranates

C. Montesinos-Herrero (1), V. Taberner (1), M. del Río (1), A. Guardado (1), L. PALOU (1)
(1) IVIA, Postharvest Technology Center, Montcada, València, SPAIN
Phytopathology 100:S86

Spain is the first European Union producer and exporter of pomegranates (*Punica granatum* L.) and 'Mollar de Elche' is the most important cultivar. Studies show remarkable benefits from pomegranate consumption on human health mainly due to the high antioxidant activity of this fruit. As new markets based on the manufacture of derived functional food products are arising, longer storage life of fresh pomegranates is demanded. Decay due to gray mold, caused by *Botrytis cinerea*, is one of the most important factors limiting storability of pomegranates. In Spain, no postharvest chemical treatments are permitted and alternative antifungal methods are required. In this work, pomegranates cv. 'Mollar de Elche' were artificially inoculated with *B. cinerea* and exposed 24 h later to air (control), 95 kPa CO_2, or 30 kPa O_2 + 70 kPa CO_2 at 20°C and 90% RH for 48 h, and subsequently stored at either 20°C for 12 days or 5°C and 90% RH for 2.5 months. On pomegranates incubated at 20°C, exposure to 95 kPa CO_2 reduced gray mold incidence by 97 and 63% compared to control fruit after 2 and 5 days, respectively. However, this reduction gradually decreased along the incubation period. Decay incidence was not significantly reduced on pomegranates treated with the mixture 30 kPa CO_2 + 70 kPa CO_2. On pomegranates stored at 5°C, both gaseous shocks reduced gray mold incidence by 80 and 40% after 12 and 26 days of storage, respectively, but the treatments lacked persistence.

Evaluation of nematicides for the management of *Rotylenchulus reniformis* across management zones created using soil electrical conductivity

S. R. MOORE (1), K. S. Lawrence (1), B. V. Ortiz (1), J. N. Shaw (1), J. Fulton (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S86

Management zones delineated by soil electrical conductivity and nematode populations are currently being examined as an option for control of *Rotylenchulus reniformis* on cotton. A trial to evaluate differences in the effectiveness of selected nematicides within each zone was established in a 26 hectare field in south Alabama in 2009. The field was delineated into low, medium, and high risk zones, with soils ranging from sandy loam to silt clay and initial populations of *R. reniformis* averaging 134, 746, and 1,775 per 150 cm3 of soil, respectively. The nematicide treatments of Telone II (1, 3-dichloropropene) 28 L/ha and Temik 15G (aldicarb) 3.9 kg/ha increased ($P < 0.1$) the total number of bolls per plant in the high risk zone compared to the untreated control. All nematicides increased the average weight per boll in the high risk zone by an average of 1.75 g, with the treatments of Avicta Complete Pak + Vydate CL-V 1.2 L/ha and Avicta Complete Pak +Temik 15G 3.9 kg/ha + Vydate CL-V 1.2 L/ha significantly ($P < 0.1$) increasing average weight per boll. Within the medium risk zone cotton treated with nematicides produced a comparable number of bolls per plant and average weight per boll compared to the untreated control. Nematicides in the low risk zone also produced a total boll number comparable to the untreated control, however average weight per boll was increased ($P < 0.1$) by Telone II 14 L/ha over the untreated control.

Histopathology of *Colletotrichum acutatum* on citrus leaves

S. G. MORAES (1), N. A. Peres (2), N. S. Massola (3)
(1) University of Sao Paulo, Piracicaba, BRAZIL; (2) University of Florida, Wimauma, FL, U.S.A.; (3) University of Sao Paulo / ESALQ, Piracicaba, BRAZIL
Phytopathology 100:S86

Colletotrichum acutatum is the causal agent of two citrus diseases, postbloom fruit drop (PFD) and Key lime anthracnose (KLA). The infection process of *C. acutatum* was studied using scanning electron microscopy (SEM). Detached leaves of sweet orange and Key lime were inoculated with suspension of 10^5 conidia/mL in a previously marked area. Isolates FSH-CLB-2 and KLA-CRD-CV-1, collected respectively from sweet orange flowers and Key lime anthracnose-affected leaves, were used. Inoculated leaves were incubated in humid chambers at 25°C during continuous wetness periods of 24, 48, 72, 96 and 120 h. Afterward, inoculated tissues were removed and processed for the visualization by SEM. Secondary conidia of both isolates were observed 24 h after inoculation on sweet orange and Key lime leaves. These conidia originated from hyphae or primary conidia. Acervuli were observed 72 hours after inoculation (h.a.i) on sweet orange leaves inoculated with the FSH-CLB-2 and KLA-CRD-CV-1 isolates. On Key lime leaves, acervuli were observed 96 h.a.i when inoculated with the KLA-CRD-CV-1 isolate.

Infected fruit as source of inoculum and infection dynamic on olive anthracnose caused by *Colletotrichum acutatum*

J. Moral (1), M. VICENTE (1), A. Trapero-Casas (1)
(1) Universidad de Cordoba, Cordoba, SPAIN
Phytopathology 100:S86

Anthracnose of olive (*Olea europaea*) caused by *Colletotrichum* species is the most serious fruit disease of this crop. Mummified fruit are the main inoculum source for olive anthracnose. However, effect of weather conditions on inoculum production by mummified fruit has not been studied. Mummified fruit were independently incubated at several temperatures (from 5 to 35°C) for 72 h and various wetness periods (from 0 to 168 h) at 22°C. Conidial production reached the maximum at 20°C. Conidial production increased linearly with wetness period from 0 to 96 h, although there was an important reduction at 168 h. When mummified fruit were subjected to successive incubation/washing treatments, conidial production decreased exponentially with the number of treatments. On rotted and non-mummified fruit, the pathogen produced significantly more conidia on the susceptible cvs. Hojiblanca (4.7×10^5 conidia/mm^2) and Picudo (4.2×10^5 conidia/mm^2) than on resistant cv. Picual (2.6×10^5 conidia/mm^2). Under field conditions, mummies placed on the olive canopy produced a high number of conidia during all year with peaks occurring in early fall. However, conidial production was greatly reduced when mummies were placed on the soil

surface or buried. Disease progress on infected fruit over time was faster for the susceptible cvs. Hojiblanca and Picudo than for the resistant cv. Picual. Besides, there was a positive correlation between rainfall and disease severity for all cultivars during the three years of study.

Late blight resistance assessing of a segregating population of diploid potatoes (*Solanum phureja*)

J. G. MORALES (1), B. Franco (2), C. E. Ñústez (3), J. M. Cotes (2)
(1) Universidad Nacional de Colombia sede Medellín, Depto. Ciencias Agronómicas, Medellin, COLOMBIA; (2) Universidad Nacional de Colombia, Medellín, COLOMBIA; (3) Universidad Nacional de Colombia, Bogotá D.C., COLOMBIA
Phytopathology 100:S87

Potato late blight caused by the oomycete *Phytophthora infestans* is the most important disease of this crop. Several chemical applications are required every crop season to achieve effective disease control. Plant resistance is the most effective strategy for disease control. *Solanum phureja* is a cultivated diploid potato from South America and an important source of late blight resistance. At Universidad Nacional de Colombia a breeding programme has been established looking for resistant potato varieties. 500 clones from a diploid *S. phureja* segregant population obtained from a cross between one resistant and one susceptible genotype were tested for late blight resistance using the Area Under The Disease Progress Curve (AUDPC) relative to the susceptible control method. The experiment was performed during two different time periods. 22 genotypes showed equal or higher values than the susceptible control indicating high disease pressure during the evaluation time period. 31 genotypes scored a value of 0 indicating immunity. Intermediate values ranging from 0.6 to 7.3 were found for the remaining genotypes. These results suggest that several genotypes within the *S. phureja* collection are important sources for late blight resistance and may be used for potato breeding against this important disease.

Isolation and characterization of *P. chlamydospora* from grapevines in Mexico

L. G. Morales-Pedraza (1), R. HERNANDEZ-MARTINEZ (1), C. Valenzuela-Solano (2)
(1) Microbiology Department, CICESE, Carretera Ensenada-Tijuana No. 3918 Zona Playitas, Ensenada, Baja California, MEXICO; (2) INIFAP, Campo Experimental Costa de Ensenada, Ensenada, Baja California, MEXICO
Phytopathology 100:S87

Grapevine is the most important fruit crop in Ensenada, Baja California, Mexico. Over the last few years an increasing number of vineyards showing decline, vascular discoloration and dead of spurs, arms, and cordons had been found in this region. In a survey conducted from 2007–2009, six isolates of *Phaeomoniella chlamydospora* were obtained from young grapevines showing Petri disease symptoms and ten from old vines showing esca in five different localities. The infected cultivars were Cabernet Sauvignon, Merlot and Mission. Trunks and cordons from these grapevines showed, in lengthwise-section brown wood streaking, and in cross-section black spots in a continuous ring around the central pith. The pathogen was also isolated from the graft union of some plants. Isolates were identified based on a previous morphological description charts and by internal transcribed spacer (ITS1-5.8S-ITS2) rDNA sequences. To our knowledge, this is the first report of *P. chlamydospora* on grapevine in Mexico.

Spatial analysis of lethal chlorosis cucurbits caused by *Zucchini lethal chlorosis virus* (ZLCV)

A. S. Moreira (1), C. R. Costa (1), J. C. Barbosa (1), A. B. FILHO (1), J. A. Rezende (1), J. S. Giampan (2)
(1) Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de São Paulo, Piracicaba, BRAZIL; (2) Instituto Agrônomico do Paraná, Londrina, BRAZIL
Phytopathology 100:S87

The lethal chlorosis of cucurbits, caused by Zucchini lethal chlorosis virus (ZLCV), has been an important viral disease that can occur in high incidence and can cause severe yield damages specially on zucchini squash crops. It is transmitted by the thrips Frankliniella zucchini. Four experiments were conducted between December 2006 and January 2009 in order to study the spatial distribution of the disease. Each experiment was conducted by using 300 plants and which were evaluated every 3 days. Disease incidence was observed by the presence of symptoms and later confirmed by serological test PTA-ELISA. Forty one assessments were carried out over the 4 trials. The parameters of Taylor's power law suggested random distribution of the disease and the values of dispersion index were significantly equal to 1.0. This result indicates the predominance of primary infection on the field, and probably the pathogen migrated from alternative host plants carried by viruliferous thrips.

Alteration of cytokinin biosynthesis by *Ustilago maydis*: Impacts on pathogenesis

E. N. MORRISON (1), R. Emery (1), B. J. Saville (1)
(1) Trent University, Peterborough, ON, CANADA
Phytopathology 100:S87

Common smut of corn, caused by *Ustilago maydis*, is characterized by the appearance of tumors that form on the aerial portions of the plant. Early studies identified increased cytokinin [CK]-like activity associated with these tumors. However, the role of *U. maydis* CK biosynthesis during infection has not been thoroughly investigated. We created solopathogenic strains (SG200) of *U. maydis* in which the sole tRNA-isopentenyltransferase (*tRNA-IPT* gene) has been deleted. The first and rate-limiting step in cytokinin biosynthesis in plants is catalyzed by isopentenyltransferases (IPTs). In fungi, related IPTs are usually tRNA-isopentenyltransferases (tRNA-IPTs). We determined, by liquid-chromatography-electrospray ionization-tandem mass spectrometry, LC- (ESI) MS/MS, that none of the major CKs produced in wild type cultures are detectable in the tRNA-IPT deletion mutant strains. These strains have different disease development profiles than wild type strains. Further investigation of the roles of CK production by *U. maydis* in disease development was investigated through deletion of the tRNA-IPT gene in compatible haploids and over expression of the *tRNA-IPT* gene in solopathogens and compatible haploids. A comparison of CK production and pathogenic development by these strains will be presented.

Distribution and prevalence of strains of *Potato virus Y* (PVY) in North Western Iran as determined by RT-PCR

L. MOUSAVI (1), J. Mozafari (1), F. Rakhshandehroo (2), S. Ghadamyari (3), N. Sokhandan (3)
(1) Department of Genetics and National Plant Gene-Bank, Seed and Plant Improvement Institute, Karaj, IRAN; (2) Department of Plant Pathology, Islamic Azad University, Science and Research Branch, Tehran, IRAN; (3) Department of Plant Pathology, Tabriz University, Tabriz, IRAN
Phytopathology 100:S87

In order to survey distribution and prevalence of strains of *Potato virus Y* (PVY) in North Western Iran 381 symptomatic infected samples were collected from potato fields of this main potato growing region during the years 2007 and 2008. Collected samples were first tested for PVY infection using a double antibody sandwich enzyme linked immune-sorbent assay (DAS-ELISA) technique. Seventy nine samples (20.73% of collected samples) were tested positive for PVY infection. The highest level of infection was observed In Gilak-Abad district of Sarab County, while the lowest infection of the virus was observed in Oughan district in suburb of Sarab City. RT-PCR detection of PVY strains using specific primers resulted in amplification of DNA fragments of 725bp, 1553bp, 352bp, and 616bp specific to PVY strains NTN, C, O, and N, respectively. The highest strain diversity in PVY was detected in Shirehjin district of Sarab County and the lowest in Ghaleh Jugh district of Bostan-Abad County. Both infection types of single and multiple infections of PVY stains were observed in the region. Out of 79 PVY infected samples 77.21%, were infected with strain O, 62.02% strain C, 39.24% strain N and 8.86% with strain NTN. The highest level of multiple infections was observed for combination of strains C+O (27.84%) and the combination of triple strains O+N+C (15.18%). This is the first report on the detection of the PVY strain NTN in North Western Iran.

Integrated management of Fusarium crown rot of wheat using fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance

E. A. MOYA (1), A. T. Dyer (1), B. J. Jacobsen (1)
(1) Montana State University, Bozeman, MT, U.S.A.
Phytopathology 100:S87

Fusarium crown rot (FCR) of wheat (*Triticum aestivum* L.) is a perennial problem for wheat producer worldwide. In glasshouse trials, difenoconazole (0.65 g/100 g seed) (Dividend-Syngenta) fungicide seed treatment reduced FCR severity by 29.3% compared to the control on cultivar Hank. The cultivar Volt had the highest innate activity levels of three pathogenesis-related (PR) proteins in apoplastic fluids among five non-inoculated spring wheat cultivars and the lowest disease severity ($P < 0.05$). Induction of systemic acquired resistance (SAR) with foliar applications of *Bacillus mycoides* isolate BmJ (1.5×10^8 cfu/ml) or acibenzolar-*S*-methyl (1.0mM) (ASM [Actigard-Syngenta]) on the cultivars Hank, Knudson and Volt reduced FCR severity by 10% compared to a control ($P < 0.05$). BmJ application increased concentrations of peroxidase and chitinase, while ASM increased β-1, 3-glucanases levels in Volt and Hank compared to water controls ($P < 0.05$). Integration of the management tools: difenoconazole seed treatment, cultivar resistance, and SAR induction, showed integration of all of them did not reduce disease severity more than use of cultivar resistance plus fungicide seed treatment or SAR induction in greenhouse trials. In a dryland field trial, integration of all three management tools reduced disease severity and FCR

populations more than individual tools ($P < 0.05$), while in an irrigated field trial SAR induction with BmJ provided similar control to difenoconazole.

Seasonal ascospore release by *Erysiphe necator* and impact upon epidemic severity of grape powdery mildew

M. M. MOYER (1), D. M. Gadoury (1), W. F. Wilcox (1), R. C. Seem (1)
(1) Cornell University NYSAES, Geneva, NY, U.S.A.
Phytopathology 100:S88

Differences in quantity of ascosporic inoculum and in-season weather can cause substantial year-to-year variation in severity of epidemics of grape powdery mildew (*Erysiphe necator*). Studies of established disease foci in 2008 and 2009 demonstrated that when focal intensity was below a threshold level, severity of fruit infection was directly proportional to time of establishment. However, when focal intensity is high, even late establishment led to severe fruit disease. To assess variable availability of ascosporic inoculum, cleistothecia from NY, NJ, WA, NC, GA, and VA were collected and overwintered at collection sites or in NY in 2006–2010. In lab assays from January to June, ascospores were released as early as February, and between 51% and 98% of the season total ascospores were released before grapevine budbreak, indicating possible release if conducive events (rain > 2.5 mm coincident with > 10C) occurred in the field. Locally, from 3 to 10 conducive events were recorded in 2000–2009 prior to budbreak. Subsequent analysis showed that the date of inoculum depletion was related to spring degree day accumulation, i.e., warmer temperatures resulted in earlier depletion dates. Model development for estimating inoculum load and results of volumetric spore trap studies will be discussed.

Surveys for Tomato ringspot virus in central Maryland vineyards

W. MSIKITA (1), K. A. Rice (1)
(1) Maryland Dept. of Agriculture, Annapolis, MD, U.S.A.
Phytopathology 100:S88

A study was initiated to confirm the presence of Tomato ringspot virus (ToRSV) in central Maryland vineyards, and to test the sensitivity of a commercially available enzyme-linked immunosorbent assay (ELISA) kit to detect the virus. Initial surveys covered 9 commercial vineyards in four counties (Baltimore County, Carroll, Frederick, and Montgomery). In the field, grape plants were visually inspected for characteristic symptoms of ringspost, leaf mottling, stunting, reduction in fruit size, abortion of the berries, and death. Suspected plants were sampled and tested in ELISA. Based on ELISA test analyses, a commercial vineyard in Carroll county was selected for intensive diagnostic surveys that included quantification of *Xiphinema* spp. nematodes in soil samples, and detection of the virus by ELISA and the reverse-transcription polymerase chain reaction (RT-PCR) in nematodes, grape and dandelion hosts. ELISA consistently was unable to detect the virus in 76 grape samples from the 9 vineyards, but detected the virus in dandelion and nematode samples from a Carroll county vineyard. RT-PCR was able to detect the virus in all three types of samples. Of the 967 nematodes extracted from a composite soil sample, 278 (28%) were *Xiphinema* spp., and of the 51 dandelion leaf samples, 3 (6%), tested positive to the virus. The sensitivity of ELISA to detect ToRSV in grapes is discussed with a view to raise awareness of this important detection tool in ToRSV disease certification.

Defense related enzymes and gene expression after resistance induction by rhizobacteria and silicon against Ralstonia solanacearum in tomato

H. K. MULAT (1)
(1) Gottfried Wilhelm Leibniz University of Hannover, Hannover, GERMANY
Phytopathology 100:S88

Bacterial wilt caused by Ralstonia solanacearum is one of the most destructive diseases in tomato production. Silicon and rhizobacteria were tested in single and simultaneous application to elicit active defense responses in tomato against this pathogen. Individual application of silicon and rhizobacteria significantly reduced bacterial wilt incidence by 50.7 and 26.8% in tomato genotypes KK2 (moderately resistant) and L390 (susceptible) (silicon amendment), and by 31.1, and 22.2%, respectively, (rhizobacteria application). The elicitors also reduced bacterial populations in the mid-stem of tomato but not in simultaneous application of the two elicitors. Silicon amendment significantly increased the silicon content in the roots of both genotypes but not in the stem, which is typical for silicon non-accumulator plants. Non-significant increases of peroxidase and phenylalanine ammonia lyase activity were observed in the individual treatments of silicon and rhizobacteria upon inoculation with R. solanacearum, while the activity of lipoxygenase was significantly decreased in the pathogen inoculated silicon amended, but increased in the rhizobacteria treatment. In simultaneous application of silicon-rhizobacteria, the activity of the three enzymes was significantly reduced. To elucidate the molecular mechanisms underlying silicon-rhizobacteria mediated induced resistance, results of transcriptome analysis of up and down regulated genes will be presented.

Association of '*Candidatus* Liberibacter solanacearum' with psyllid-affected carrots in Europe

J. E. Munyaneza (1), T. W. Fisher (2), V. SENGODAGOUNDER (3), S. F. Garczynski (1), A. Nissinen (4), A. Lemmetty (4)
(1) YARL, USDA-ARS, Wapato, WA, U.S.A.; (2) Department of Entomology, Washington State University, USDA-ARS, Wapato, WA, U.S.A.; (3) Department of Plant Pathology, Washington State University, YARL, USDA-ARS, Wapato, WA, U.S.A.; (4) Agrifood Research Finland, Plant Protection, Jokioinen, FINLAND
Phytopathology 100:S88

Carrot psyllid (*Trioza apicalis*) is a serious pest of carrots (*Daucus carota*) in northern and central Europe. Carrots exhibiting symptoms of psyllid damage were observed in commercial fields in southern Finland in 2008. Symptoms in affected plants included leaf curling, yellow and purple discoloration of leaves, stunted growth of shoots and roots, and proliferation of secondary roots. Given recent association of liberibacter with several crops affected by psyllids, an investigation on whether this bacterium is associated with carrots with psyllid symptoms was conducted. PCR primer pairs OA2/OI2c and LsoF/OI2c, specific for the 16S rRNA gene from "*Candidatus* Liberibacter solanacearum", generated amplicons of 1,168-bp and 1,173-bp, respectively, from DNA extracted from field-collected and laboratory-reared psyllids, and symptomatic carrots. In contrast, no PCR products were detected in DNA extracted from insect-free plants. The DNA sequences of amplicons of the genes encoding liberibacter 16S rRNA from psyllids and carrots were identical. The DNA of the 16S rRNA gene sequences determined from carrots and psyllids were over 99.9% identical to analogous sequences of "*Ca*. L. solanacearum" amplified from several solanaceous crops and the potato psyllid, vector of this bacterium. This is the first report of "*Ca.* L. solanacearum" associated with a non-solanaceous species, and the first report of this pathogen outside of North and Central America and New Zealand.

Genotyping *Xylella fastidiosa* strains using multiplex PCR

B. A. MYERS (1), J. A. Brady (2), F. L. Mitchell (2), H. B. Rathburn (1)
(1) Tarleton State University, Stephenville, TX, U.S.A.; (2) Texas AgriLife Research, Stephenville, TX, U.S.A.
Phytopathology 100:S88

Xylella fastidiosa is a gram negative, xylem-limited, nutritionally fastidious bacterium that causes leaf scorch diseases such as plum leaf scald (PLS), oleander leaf scorch (OLS), and Pierce's disease (PD) of grapevines. Four different subspecies and numerous strains of the bacterium have been recognized in North America. OLS is caused by *X. fastidiosa* subsp. *sandyi*, PD strains belong to *X. fastidiosa* subsp. *fastidiosa*, and *X. fastidiosa* subsp. *multiplex* causes PLS and a number of tree leaf scorch diseases. The recently described *X. fastidiosa* subsp. *tashke* causes leaf scorch in chitalpa trees. While each subspecies can occupy a large number of host plants, they cause disease symptoms in a very small subset of potential hosts. Genotyping the different bacterial subspecies and strains for epidemiological studies can be time consuming and expensive using currently available approaches. Additionally, current approaches lack marker density necessary for a substantive assessment of recombination. We are developing a multiplex PCR assay covering hundreds of genetic loci that will distinguish even closely related *X. fastidiosa* isolates. The assay has proven to be robust, inexpensive, and provides a highly informative genetic fingerprint that will facilitate an understanding of plant/vector/pathogen relationships.

Maize *chiA* as a potential genetic marker for *Stenocarpella maydis* ear rot resistance

T. A. NAUMANN (1)
(1) USDA/ARS/NCAUR, Peoria, IL, U.S.A.
Phytopathology 100:S88

Stenocarpella maydis (*Diplodia maydis*) is the most prevalent ear rot pathogen in nearly all countries where maize is produced. The genetic basis of plant resistance to *S. maydis* appears to rely on multiple genetic factors, none of which are known. We previously reported that *S. maydis* secretes a protein, Stm-cmp, that modifies maize ChitA, a chitinase that is produced abundantly during seed development. We also demonstrated that ChitA protein from inbred B73 is highly susceptible to Stm-cmp modification while ChitA from inbred LH82 is resistant. These ChitA proteins are encoded by alleles of the *chiA* gene that encode proteins with six polymorphisms. Here I report cDNA cloning of both *chiA* genes, construction of yeast strains that produce the ChitAs, purification of yeast-produced ChitAs, and their in vitro modification by fungal St-cmp. In addition, I created yeast strains that produce mutant versions of ChitA. By comparing the susceptibility to Stm-cmp modification of the mutant ChitA proteins I determined that a single amino acid, encoded by a chiA single nucleotide polymorphism (SNP) results in resistance. This SNP may be a useful marker for breeding resistance to *S. maydis* ear rot.

Effects of seed treatment on root diseases and yields of soybean
S. S. NAVI (1), X. Yang (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S89

The objective of the study was to evaluate the effects of fungicide seed treatment on sudden death syndrome (*Fusarium virguliforme*), Rhizoctonia root rot (*Rhizoctonia solani*), and Sclerotinia stem rot (*Sclerotinia sclerotiorum*) and their effects on soybean yields. Field tests against *F. virguliforme* and *R. solani* was conducted at Ames, and against *S. sclerotiorum* at Nashua, during 2005 to 2009. Products from various companies and an unregistered bio-fungicide from our lab were tested. Experiments were laid out in RCBD with four replications. Percent incidence and severity due to *R. solani* was recorded at 7, 14, and 21 days after emergence and for *F. virguliforme* and *S. sclerotiorum* at flowering, pod formation and maturity growth stages. The central four rows of each plot were harvested and plot yields bushels/acre (adjusted to 13% grain moisture) was recorded. Some of the products tested were effective in minimizing the diseases and increased yields from 3 to 9 bushels per acre over untreated controls.

A predictive model for carpogenic germination of *Sclerotinia sclerotiorum*
A. NEPAL (1), L. E. del Rio Mendoza (1)
(1) Dept. Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S89

A predictive model for carpogenic germination (CG) of *S. sclerotiorum* was developed using soil moisture data. Two types of soils from Fargo (Fargo Silty clay) and Kindred (Aylmer-Bantry fine Sand), ND were mixed in proportions of 1:0, 2:1, 1:1, 1:2, and 0:1 v/v to create different textures. Sclerotia were buried in samples from each soil texture set at constant 100%, 75%, 50% or 25% soil saturation; or to conditions fluctuating back and forth between 100 to 0; 75 to 0; 50 to 0; and 25 to 0% saturation with 25% saturation intervals. Samples were incubated at 14/18°C day/night for 82 days. CG was recorded at five-day intervals. CG data were expressed as binary values using 15 and 20% CG as thresholds. This data was split into two portions, one was used for model development using logistic regression analysis and the other was left for model validation. The area under cumulative moisture curve and rate of moisture accumulation were calculated for every time interval in all treatments and used, along with percentage of clay and silt, as predictor variables for the model. The best model had $c = 0.96$. When the model was validated using the independent data set, it produced a true negative proportion of 73% and a true positive proportion of 100% for an overall accuracy of 85%. Field validation of this model will be discussed.

Brown girdling root rot as a potential threat to canola production in North Dakota
A. NEPAL (1), L. E. del Rio Mendoza (1)
(1) Dept. Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S89

Rhizoctonia solani was observed causing brown girdling root rot of canola plants in research plots in Langdon, ND in 2008 and 2009. Since *R. solani* is known to cause important yield reductions in other canola producing areas of the world, a study was conducted to assess the potential threat this pathogen represents to the canola industry in ND. To evaluate the protection provided by conventional seed treatment, seeds from four commercial canola cultivars were washed in running water to eliminate the chemicals from the seeds. Washed and non-washed seeds were planted in replicated trials in greenhouse soil mix infested or not with canola seeds colonized by an *R. solani* AG 4 isolate. Pots were maintained at 21°C and 14 hours light daily. Germination and plant standing were quantified 14 and 20 days after planting, respectively. The experiment was repeated once. The seed treatment did not increase germination nor plant standing significantly ($\alpha = 0.05$). Germination and plant standing were reduced from 90 to 70% and from 87 to 57%, respectively, when seeds were planted in *R. solani*-infested soils. Cultivars IX08-7121R and Hyclass 712 had significantly less germination (mean of 64%) than cultivars DKL30-42 and G75449 (mean of 77%). To get a better estimate of the potential impact of this disease on the canola industry in North Dakota, a larger study involving 59 commercial cultivars and *R. solani* isolates from three AG groups (4, 2-1, and 2-2) is under way.

Effects of temperature and wetness duration on sporangia germination and infection of cucurbit varieties by *Pseudoperonospora cubensis*
K. NEUFELD (1), P. Ojiambo (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S89

Cucurbit downy mildew caused by *Pseudoperonospora cubensis* is considered the most damaging disease of cucurbitaceous crops worldwide. Recently, we develop a model to quantify the combined effects of temperature (t) and leaf wetness duration (w) on sporangia germination and infection by *P. cubensis* using cantaloupe. However, the influence of host type on predictive ability of the model has not been determined. The objective of this study was to determine the effect of host type on the infection parameters of *P. cubensis* to combined effects of t and w. Three cucurbit types (cantaloupe, cucumber and squash) were inoculated with *P. cubensis* and exposed to a range of leaf wetness durations (2–24 h) and fixed temperatures (5–30°C) in growth chambers. Germination was assessed at the end of each wetness period and infection was recorded at 5 and 7 days after inoculation. Data were fitted to a Weibull function of the form $f(w,t) = f(t)\bullet(1\text{-}\exp\{\text{-}[B \times w]^D\})$. Host type, t, w, and t × w significantly ($P < 0.05$) affected infection parameters. Optimum range of temperature for the infection parameters was found to be between 10–25°C. Infection parameters had a minimum leaf wetness duration of 8 h with broader optimums with increasing wetness. Host types varied in their response to w at a given t. Host based nomograms were developed to predict the potential risk of cucurbit downy mildew epidemics based on observed or forecasted temperature and leaf wetness duration.

Analysis of transgenic American chestnut
A. E. NEWHOUSE (1), A. B. Zhang (1), L. Northern (1), C. A. Maynard (1), W. A. Powell (1)
(1) SUNY-ESF, Syracuse, NY, U.S.A.
Phytopathology 100:S89

Chestnut blight (caused by *Cryphonectria parasitica*) is a classic example of the devastating impact an invasive pathogen can have on a well-established native tree species. One strategy to combat this disease is to modify the host by adding one or more new genes to enhance disease resistance. Several lines of transgenic American chestnut (*Castanea dentata*) have been produced through Agrobacterium co-transformation of somatic embryos. Co-transformation allows constructs containing a marker gene and a putative resistance-enhancing gene to be inserted independently within the genome of the host. Selection for co-transformed events is both visual (i.e. GFP) and physiological (antibiotic resistance). PCR confirms the presence of both genes early in the regeneration process, and once individual transgenic lines are established in tissue culture, they are multiplied and regenerated into whole plants. Subsequent analyses (Southern hybridization, quantitative real-time PCR, or both) have determined transgene copy number for all established lines. Of five initial lines, three had a single copy of the putative resistance-enhancing gene, one had two copies, and one had three copies. Transgene expression has been analyzed with a colorimetric enzyme assay (oxalate oxidase) and with real-time PCR. Greenhouse and laboratory tests are also underway to screen immature trees for disease resistance based on inoculations with *C. parasitica*.

Causal organisms of black spot on postharvest rambutan in Mexico
D. NIETO (1), M. G. Hernandez (1), D. Teliz (1), C. Nava (1), M. T. Martinez (2), N. Bautista (1)
(1) Colegio de Postgraduados, Texcoco, MEXICO; (2) Universidad Autonoma Chapingo, Texcoco, MEXICO
Phytopathology 100:S89

The rambutan is a tropical fruit introduced to Mexico. It's marketing is limited by a short shelf life and a postharvest disease which is a dark brown spot in the pericarp. The aim of this study was to identify the causative agent of black spot in postharvest rambutan. Pieces of infected pericarp were disinfested for 2 min in 2% NaOCl, plated on potato dextrose agar and incubated for 7 days. One of the fungi was white uniform and radial growth with acervuli. The second fungi showed abundant black mycelium and pycnidia. For replication of symptoms, 60 fruit were disinfested 2 min in 2% NaOCl. After, these were inoculated by smitten with a drop of spore solution (1×10^3 spores/ml) and placed in a moist chamber for 7 days. The symptoms appeared after 3 days and on the sixth day developed abundant mycelium. Reisolated fungi were identical to the originals. The first fungus had 5 cells, the basal and apical were hyaline, while the intermediate two were brown. There were three appendices in the apical and basal cells. The second fungus forms black conidia, bicellular, both cells with a nucleus. The fungi were identified as *Pestalotiopsis thea* and *Lasiodiplodia theobromae* based on characteristics of conidia and molecular analysis (GenBank accession AY681477.1 and FJ478102.1). *Lasiodiplodia* sp. and *Pestalotiopsis* sp. already been reported in other countries where it is grown rambutan. However, this is the first report of *L. theobromae* and *P. thea* causing black spot and fruit rot on rambutan in Mexico.

Curtoviruses in leafy greens in Arizona
C. NISCHWITZ (1)
(1) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S89

Curtoviruses are transmitted by the beet leafhopper (*Circulifer tenellus*) and can cause severe losses in sugar beets and vegetable crops in the western U.S. In spring 2009, spinach in southern Arizona showed severe curly top-like

symptoms and numerous beet leafhoppers were observed in the field. Samples submitted to a commercial diagnostic lab were negative for curtoviruses in a general curtovirus PCR screen. However, using primers developed in our lab, a curtovirus, *Pepper curly top virus*, was detected in symptomatic spinach. As a result of the failed detection by a commercial lab, a survey was conducted on leafy greens including spinach and table beets, weeds and crops near leafy green fields to determine which curtoviruses are present and if they are established in Arizona. Fields in four locations in Arizona were selected and samples were collected twice in each field throughout the season. DNA was extracted from the samples and tested for curtoviruses using PCR. The PCR products of selected samples were sequenced. One or more samples of spinach, table beets and chard in all locations tested positive for curtoviruses. Reported weed hosts *Sisymbrium irio* and *Chenopodium sp*. and a new weed host *Funastrum hirtellum* were identified. Perennial alfalfa in the vicinity of some spinach fields also tested positive. These findings indicate that curtoviruses are established in the farmscape in Arizona, and curly top disease in leafy greens is caused by various curtoviruses.

Analysis of rice *CHROMOMETHYLASE 3* (*OsCMT3*) in RNA silencing mediated by geminivirus

M. NISHIGUCHI (1), Y. Suizu (1), H. Chen (1), N. Yamaoka (1)
(1) Ehime University, Matsuyama, JAPAN
Phytopathology 100:S90

Chromomethylase (CMT) is one of three classes of cytosine methylatransferase in Arabidopsis. CMT3 is preferentially involved in cytosine methylation in CpNpG sequences. Based on the nucleotide sequence of *CMT3*, we selected a rice mutant where a retrotransposon *Tos17* was inserted in a rice homolog of *CMT3* (*OsCMT3*). In the mutant (*oscmt3*), RNA silencing induction was examined by particle bombardment with a plasmid expressing *GFP* (p35S-GFP) in combination with a silencing inducer, pGFP RNAi or pWI-GFP RNAi (recombinant *Wheat dwarf geminivirus*), each carrying inverted repeat (IR)-DNA of *GFP* sequence. When rice tissues were bombarded with the mixture of p35S-GFP and pGFP RNAi, interestingly, *GFP* expression was reduced in *OsCMT3* but not in oscmt3, implying that *OsCMT3* is required for IR-DNA induced silencing. When the double-stranded RNA of in vitro transcripts from GFP DNA was used instead of pGFP RNAi, *GFP* expression was reduced in both *OsCMT3* and *oscmt*3. This suggests that *OsCMT3* functions at the level of DNA before transcription. When we bombarded with the mixture of pWI-GFP RNAi and p35S GFP, GFP expression was also reduced in both rice types. These results suggest that *OsCMT3* is indispensable for IR-DNA induced silencing but not for that mediated by *Wheat dwarf geminivirus.*

A survey for grapevine viruses in Virginia vineyards

M. NITA (1), T. Mekuria (2), N. A. Rayapati (3)
(1) Virginia Tech, Winchester, VA, U.S.A.; (2) Washington State University, IAREC, Prosser, WA, U.S.A.; (3) Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S90

Commercial vineyards in the commonwealth of Virginia were surveyed for grapevine leafroll disease (GLRD). In red-berried wine grape cultivars (*Vitis vinifera*), leaves of GLRD affected vines exhibited green veins, inter-veinal reddening and downward rolling. In white-berried cultivars, leaves of infected vines showed mild yellowing and downward rolling. During 2009 growing season, leaf samples were collected from about 500 grapevines planted in 45 vineyards. Petiole extracts were tested for Grapevine leafroll-associated virus 2 (GLRaV-2), GLRaV-3 and Grapevine fleck virus (GFkV) by one tube-single step RT-PCR using primers specific to a portion of the heat-shock protein-70 homolog (HSP70h) of GLRaV-2 and -3 and replicase gene of GFkV. Nearly 70% of the samples tested positive for one of the three viruses, with a significant majority testing positive for GLRaV-3. The proportion of positive samples containing GLRaV-3 alone was higher than those with either GLRaV-2 or GFkV. In addition, GLRaV-2 and GFkV were found as mixed infection with GLRaV-3. Majority of the vineyards that tested positive for GLRaV-2 or GLRaV-3 were planted in 80's or before. The RT-PCR fragments amplified from select number of positive samples were cloned and their sequences compared with corresponding sequences available in GenBank. Results indicated the presence of genetically distinct isolates of GLRaV-2, GLRaV-3 and GFkV in Virginia vineyards.

Specific patterns of co-occurrence of grapevine viruses in Washington vineyards

M. NITA (1), T. Mekuria (2), N. A. Rayapati (3)
(1) Virginia Tech, Winchester, VA, U.S.A.; (2) Washington State University, IAREC, Prosser, WA, U.S.A.; (3) Washington State University, Prosser, WA, U.S.A.
Phytopathology 100:S90

A survey for grapevine viruses conducted in Washington vineyards during 2005 and 2009 revealed the presence of Grapevine leafroll-associated virus 1 (GLRaV-1), -2, -3, -4, -5 and -9), Grapevine rupestris stem pitting-associated virus, Grapevine Virus A (GVA), GVB and Grapevine fanleaf virus in many wine grape cultivars. These viruses were found occurring as single and/or mixed infections in individual grapevines. In order to determine the scale of association (or lack of) of viruses in mixed infections at the individual plant level, the data sets from the survey were analyzed by the Jaccard association analysis to measure the probability of any two viruses co-occurring in individual grapevines. Results based on the Jaccard similarity index indicated that some grapevine viruses such as GLRaV-2 and -4 were significantly positively associated and others like GLRaV-1 and -2 were negatively associated. Multivariate analysis of the data sets showed that co-occurrence of GLRaV-3 and GVA, which are known to be transmitted by similar vector(s), were highly correlated. Determining the nature of an association (positive, negative, or neutral) between taxonomically disparate viruses may provide valuable information on the epidemiology of component viruses and to develop robust sanitation programs for preventing spread and mitigating negative impacts of viruses.

Macroarray detection of fungal turfgrass pathogens

E. N. NJAMBERE (1), B. Clarke (1), N. Zhang (1)
(1) Rutgers The State University of New Jersey, New Brunswick, NJ, U.S.A.
Phytopathology 100:S90

Early and accurate detection and identification of fungal pathogens is critical for turf disease management. Traditionally, diagnosticians use direct observation or culturing of specimens to identify pathogens. DNA macroarray is a new molecular tool, which offers a fast, culture-independent alternative for pathogen detection. The advantage of this technique is its high throughput compared to other detection methods. In this study, we aim to increase the array detection sensitivity. We designed a macroarray for two turf pathogens, Rhizoctonia solani and Pythium aphanidermatum. The array included 9 probes specific to each species. Positive controls and internal controls were also spotted on the array. Array sensitivity was optimized by hybridizing labeled ITS PCR products of the two target species with three sets of probes: 1) monomer oligonucleotide probes (20-25 nt), 2) dimers: two tandem repeats of the monomers (40-50 nt) and 3) dimers with an intervening poly-A between the two repeats (50-60 nt). The use of repeat sequence probes increased the array sensitivity. However, specificity was compromised when an intervening poly-A sequence was included. Therefore, dimers, without the poly-A, performed best in terms of both sensitivity and specificity. These findings will be used to develop a multiplex detection/identification system for major fungal and oomycete pathogens of turfgrasses that will facilitate early diagnosis and improved disease management.

Viability staining of naturally and artificially molded sorghum caryopses using tetrazolium violet

L. W. Noll (1), D. N. Butler (1), C. R. LITTLE (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S90

Sorghum grain mold (GM) is a yield-limiting disease characterized by damage to embryo tissues. Caryopses were obtained from naturally weathered panicles and panicles inoculated with *Fusarium thapsinum* (FT) and *Curvularia lunata* (CL) at anthesis. Caryopses were assayed for tetrazolium violet (TZ) viability, embryo damage, germination, and pathogen incidence. In naturally weathered and artificially inoculated caryopses, Tx430 (GM-susceptible) showed low whole embryo viability, whereas Sureno and Tx2911 (GM-resistant) showed higher values. Tx430 had the greatest levels of non-staining scutellum, coleoptile, plumule, and radicle tissues and highest levels of embryo damage when inoculated with either pathogen. In naturally weathered grain, TZ staining overestimated germination for some genotypes (especially Tx430), whereas TZ staining underestimated germination in most artificially inoculated material. Correlations between TZ viability and germination were positive and significant for naturally weathered and artificially inoculated caryopses. FT and *F. proliferatum* (FP) were the most commonly isolated fungi from naturally weathered grain. Correlations between FT (yellow-pigmented isolates), FP, and CL incidence from naturally weathered sorghum caryopses and TZ viability were negative and significant. This study showed that TZ viability differs in a genotype- and pathogen-dependent manner. Therefore, this approach may have utility in screening sorghum germplasm for GM resistance at the whole embryo and tissue levels.

Efficacy of ametoctradin + dimethomorph for control of *Phytophthora* species infecting ornamental plants in the Eastern United States

D. J. NORMAN (1), M. M. Benson (2), M. L. Daughtrey (3)
(1) University of Florida, Apopka, FL, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) Cornell University, Long Island, NY, U.S.A.
Phytopathology 100:S90

Efficacy of fungicide active ingredients varies against *Phytophthora* species. In ornamental plant production, multiple species of *Phytophthora* may be found within a geographic region, within a single production facility, and even within a crop species grown in one production facility. The new fungicide product Orvego contains two active ingredients, ametoctradin and dimethomorph. Orvego is a foliar and root penetrant with translaminar and locally systemic activity making it an effective control against multiple *Phytophthora* species. Preliminary tests of Orvego on both annual and perennial ornamental crops under both greenhouse and field conditions have been conducted. Results indicate that Orvego was very effective in controlling *P. cryptogea*, *P. dreschleri*, *P. cinnamomi*, *P. tropicalis*, and *P. nicotianae* species on Gerbera daisy, *Rhododendron*, English ivy, and pansy. Orvego has multiple modes of action against the pathogen and it should be an effective rotation partner with other Oomycete control products for resistance management.

Selection of transformed somatic embryos by antibiotic drench technique
L. C. NORTHERN (1), C. A. Maynard (2), W. A. Powell (2)
(1) SUNY-ESF, Jamesville, NY, U.S.A.; (2) SUNY-ESF, Syracuse, NY, U.S.A.
Phytopathology 100:S91

Co-transformation of American chestnut (Castanea dentata) somatic embryos takes approximately 16 months. To shorten the selection phase of this procedure, an antibiotic drench was added. Co-transformations were performed using a 1:5 ratio of Agrobacterium EHA 105 containing a binary vector with BAR and GFP (pGFP), plus EHA 105 carrying the laccase gene of interest and NPT2 (pESF-KB-LOE). Non-transformed embryos were treated with the same conditions as negative controls. The combination of Finale®, paromomycin, carbenicillin, and cefotaxime in solid selection media has been used successfully to select for transformed embryos. A supplementary dose of antibiotics, at the same concentration as the solid medium, was added as a drenching solution when transferring the embryos to fresh media. We hypothesize that this would allow the antibiotics to penetrate the cluster of embryo tissue better than simple absorption from the surface of solid media and that this would increase the rate of mortality of the non-transformed cells. Transformed embryos without this extra dose of antibiotics were used as controls. Extra doses were added each time that the embryos were transfer to fresh media (every 2 weeks on three consecutive transfers), by covering them with the antibiotic solution for a total of 6 hrs, and then aspirating away any remaining liquid. Preliminary results indicate that the treatment was not effective in reducing the time required to eliminate non-transformed cells.

Organic and polyethylene mulches with biofungicides for managing diseases in organic tomato production system
L. M. NYOCHEMBENG (1), R. N. Mankolo (1), S. R. Mentreddy (1)
(1) Alabama A&M University, Normal, AL, U.S.A.
Phytopathology 100:S91

Plant disease management in organic production systems is a challenge hence there is significant interest in developing effective strategies for mitigating diseases and weeds. Experiments were conducted to evaluate the individual and combined effects of spent mushroom substrate (SMS) and sudan x sorghum hybrid (SS) organic mulches and polyethylene soil bed covers with or without biofungicides on the severity of diseases in two tomato varieties. In experiment 1, the organic mulch combination SMS + SS significantly enhanced tomato fruit weight, number of fruits and weed suppression compared to SS mulch and the control. In experiment 2, there were significant differences between the polyethylene mulches white plastic, reflective silver plastic and control for number of fruits/plant and powdery mildew severity. However, the plastic mulches were better than control for fruit weight and plant height irrespective of mulch type. Although polyethylene mulch enhanced plant height and fruit yield, powdery mildew was more severe in the mulch treatments. Tomato var. 'Celebrity' outperformed var. 'Amelia' in fruit yield and showed more susceptibility to powdery mildew. Spent mushroom substrate as biofungicide under polyethylene mulch also significantly enhanced tomato fruit yield compared to the control. These results show that organic and polyethylene mulches, while ineffective in reducing powdery mildew severity, significantly enhanced tomato yield and weed suppression in organic production system.

Effects of fertility and cultivation practices on large patch disease of zoysiagrass, caused by *Rhizoctonia solani* AG 2-2 LP
K. OBASA (1), R. St. John (1), D. Bremer (1), J. Fry (1), M. Kennelly (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S91

Zoysiagrass (*Zoysia japonica* and *Z. matrella*) is a warm-season (C4) turfgrass that is appropriate for many uses in the central and southern United States. Large patch, caused by *Rhizoctonia solani* AG 2-2 LP, is the most common and severe disease of zoysiagrass and is managed primarily by fungicides. The effects of cultivation (aerification, verticutting and sand topdressing) and time of nitrogen (N) fertilization on large patch development were evaluated on inoculated plots in a split-plot design with four replications. The whole plot treatment was cultivation vs no cultivation. The subplot treatment was fertility, with either polymer-coated urea equivalent to 2 lb N per 1000 ft^2 applied as one application during summer or as split applications of urea at 1 lb N each during spring and fall. Large patch severity was assessed through patch size measurements and digital image analysis. Patch sizes were significantly reduced in 2009 but not in 2008 in cultivated plots. Cultivation and summer fertilization resulted in smaller patches compared with non-cultivation and fertilization in spring and fall. However, recovery of zoysia from large patch infection during summer was faster in non-cultivated plots, irrespective of the timing of fertilization, than in cultivated plots. Cultivation and summer fertilization could potentially reduce the fungicide volume required for large patch management.

Evaluation of 15 new zoysiagrass lines for resistance to large patch disease caused by *Rhizoctonia solani* AG 2-2 LP
K. OBASA (1), M. Kennelly (1)
(1) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S91

Fifteen new zoysiagrass lines were evaluated for resistance to large patch disease caused by *Rhizoctonia solani* AG 2-2 LP under growth chamber and field conditions in 2009. Field plots measured 5 × 5 ft and were arranged in a randomized complete block design (RCB) with three replicates. Plots were maintained at a mowing height of 0.56 inches and fertilized in May with 1 lb and July and August with 0.75 lb nitrogen per 1000 ft^2 respectively using plain urea. The center of each plot was inoculated with *R. solani*-infested oat kernels in September of 2008. Large patch development in each plot was assessed through patch size measurement and digital image analysis. Growth chamber evaluations consisted of fifteen small plastic pots of each new line and the standard cultivar 'Meyer' inoculated with 8 to 10 *R. solani*-infested oat kernels and maintained at 25°C and >95% relative humidity under a 13 h photoperiod. At 5-day intervals, three pots of each line and Meyer were removed and rated individually for disease incidence by determining the percentage of shoots with distinct water-soaked lesions on the leaf sheath. No single line offered consistently better disease resistance than Meyer under both experimental conditions. However, five lines 5313-46, 5313-71, 5321-18, 5313-34 and 5325-11 had recovery rates from the disease that were comparable to Meyer.

Towards understanding coronatine-dependent suppression of innate immunity in Arabidopsis guard cells
N. OBULAREDDY (1), S. Panchal (1), M. Melotto (1)
(1) University of Texas, Arlington, TX, U.S.A.
Phytopathology 100:S91

Recent studies have shown that stomatal pores in the leaf epidermis close as a part of the plant innate immune response against bacterial invasion of plant tissues. Counteracting this response, the plant pathogenic bacteria Pseudomonas syringae pv. tomato strain DC3000 has evolved the virulence factor coronatine, an important strategy contributing to pathogenesis. The mode of action of coronatine in plant cells has beginning to be elucidated. Two components of the coronatine receptor complex have been identified, namely COI1 (the F-box subunit of E3 ligase) and JAZ (a repressor of jasmonic acid pathway) proteins, suggesting that coronatine acts in the plant by inducing the degradation of proteins and hijacking the jasmonic acid signaling pathway. However, an unanswered question is whether coronatine induce stomatal opening using the same molecular mechanism. Here, we report that among all JAZ genes, JAZ1, JAZ2, JAZ3, and JAZ9 are induced in guard cells within 30 minutes of exposure to 60 μM coronatine. In addition, coronatine-dependent binding of COI1 and these JAZ proteins has been demonstrated using yeast-two-hybrid system. We have developed single and multiple knock out plants for these genes. The phenotype of these plants and the biological significance of coronatine action in the guard cell will be further discussed.

SoilGard 12G (*Gliocladium virens* strain GL-21): A solution for controlling lettuce drop (*Sclerotinia minor/sclerotiorum*) in conventional and organic systems
S. C. OCKEY (1)
(1) Certis USA, Yakima, WA, U.S.A.
Phytopathology 100:S91

Lettuce drop is one of the most frequent and destructive diseases encountered by commercial lettuce growers throughout the U.S. The causal agents *Sclerotinia minor & S. sclerotiorum* produce sclerotia requiring long rotations and annual fumigation in fields where the disease has become established. Heavily infested fields have incurred losses of up to 70%. SoilGard® 12G Microbial Fungicide contains spores of *Gliocladium virens* strain GL-21 which has proven to be an effective fungicide in laboratory and field studies.

SoilGard® 12G was tested under field conditions in Greenfield, CA during 2009. The trial was set up as a randomized complete block design with 4 replicate plots/treatment each 2 rows x 6m. SoilGard was applied at 4.48 Kg/Ha a total of three times with a CO_2 pressurized backpack sprayer operating at 701-795 L/Ha and 276KPa. The initial application was made 1 day post planting followed by the two additional applications at approx. 4 week intervals. Disease incidence was rated throughout the trial and compiled at harvest. SoilGard 12G and the commercial standard of Endura® Fungicide at 11 oz/acre resulted in 7% disease incidence each which was significantly ($p = 0.05$) lower than the incidence for the untreated check with 23% incidence. Trials are planned to evaluate alternate application types with additional crops sensitive diseases caused by *Sclerotina* sp.

Effect of multiple virus infections on seed transmission in cowpea

K. E. OGUNSOLA (1), C. A. Fatokun (2), C. O. Ilori (3), P. Lava Kumar (2)
(1) International Institute of Tropical Agriculture (IITA), and Crop Protection and Environmental Biology Department, University of Ibadan, Ibadan, NIGERIA; (2) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA; (3) Crop Protection and Environmental Biology Department, University of Ibadan, Ibadan, NIGERIA
Phytopathology 100:S92

Multiple viral infections occur naturally in cowpea and these viruses may interact synergistically causing a change in virus concentration of one or all of the viruses. We studied the effect of multiple virus infections on virus seed-transmission in eight cowpea genotypes that differ in relative susceptibility to viruses. *Blackeye cowpea mosaic virus* (BlCMV, genus *Potyvirus*), *Southern bean mosaic virus* (SBMV, genus *Sobemovirus*) and *Cucumber mosaic virus* (CMV, genus *Cucumovirus*), all of which were known to be seed-transmitted in cowpea, were used to mechanically inoculate test plants at seedling stage singly and in all possible combinations. Seeds were harvested at maturity and assessed for seed-transmission by grow-out tests and enzyme-linked immuno-sorbent assay. Seed-transmission was found to be genotype specific and also influenced by co-infection. In cowpea cv. IT98K-133-1-1, BlCMV was not seed-transmitted either singly or in any combinations. Seed-transmission of SBMV was not observed in single infections, however, seed-transmission was detected in plants co-infected with CMV, but not BlCMV. CMV was seed transmitted in single infections as well as mixed infection with BlCMV and CMV. This study suggests possibility of synergistic interaction between co-infected viruses in facilitating seed-transmission of apparently non-seed transmitted viruses, in this case SBMV, with CMV acting as a helper virus.

A tomato 14-3-3 protein (TFT7) positively regulates immunity-associated programmed cell death mediated by diverse disease resistance proteins

C. OH (1), G. B. Martin (2)
(1) Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.; (2) Boyce Thompson Institute for Plant Research and Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S92

Immunity-associated programmed cell death (PCD) is triggered when a plant resistance (R) protein recognizes a corresponding pathogen effector protein. For example, the tomato R protein Pto recognizes the *Pseudomonas syringae* effector AvrPto causing localized PCD that is associated with disease resistance. Previously, we reported that both MAPKKKα (mitogen-activated protein kinase kinase kinase) and the tomato 14-3-3 protein 7 (TFT7) positively regulate Pto-mediated PCD in tomato and *Nicotiana benthamiana*. Moreover, we reported that TFT7, unlike MAPKKKα, is required for PCD mediated by four other R proteins. We therefore investigated why TFT7 is broadly required for PCD induced by R proteins in plants. We discovered that a MAP kinase kinase (MAPKK), that acts downstream of MAPKKKα, also interacts with TFT7 in yeast and plant cells. Gene silencing experiments revealed that the MAPKK and TFT7 are each required for PCD induced by the same set of R proteins. We will discuss further how TFT7 regulates this MAPKK for induction of PCD in *N. benthamiana*.

***In vitro* assessment of *Sclerotinia homoeocarpa* resistance to fungicides and plant growth regulators**

C. Ok (1), J. T. POPKO (1), K. Campbell-Nelson (1), G. Jung (1)
(1) University of Massachusetts, Amherst, MA, U.S.A.
Phytopathology 100:S92

Dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Bennett) is a turfgrass disease primarily controlled by fungicide application on golf courses; however, resistance has been confirmed in three of the five fungicide classes commonly used to control dollar spot. The main objective of this study was to evaluate *S. homoeocarpa* resistance to multiple fungicide classes and plant growth regulators (PGRs) and cross-resistance among active ingredients of the same class. Sixty-four isolates were randomly selected and assayed for *in vitro* fungicide sensitivity to six demethylation inhibitor (DMI), two dicarboximide, one anilene, one benzimidazole fungicide and three type II plant growth regulators. All active ingredients from the DMI class were highly correlated ($P < 0.0001$) to each other as well as to the dicarboximide (iprodione) and plant growth regulators (flurprimidol and paclobutrazol). EC50 values of all active ingredients assayed except for boscalid were significantly higher in isolates resistant to thiophanate-methyl than sensitive isolates. Results indicate that multiple class and cross-resistance of fungicides and PGRs has developed in *S. homoeocarpa* and that PGRs have a fungistatic effect similar to that of demethylation inhibitor fungicides. The high correlation of *in vitro* sensitivities among PGRs and DMI fungicides further suggest that PGRs may contribute to the selection of DMI resistant isolates or facilitate decreased sensitivity to DMI fungicides.

Assessment of SIMBLIGHT1 and SIMPHYT1 models for prediction of *Phytophthora infestans* oubreak in North-Eastern U.S. from 2004 to 2009 seasons

M. OLANYA (1), C. Honeycutt (1), R. P. Larkin (1), Z. He (1)
(1) USDA-ARS, NEPSWL, Orono, ME, U.S.A.
Phytopathology 100:S92

Accurate prediction of *Phytophthora infestans* outbreak during a cropping season is crucial for effective management of late blight. The SIMBLIGHT1, SIMPHYT1, and modified SIMPHYT1 models were assessed for prediction of late blight outbreak relative to the NOBLIGHT model based on climatic data from field experiments. The dynamics of late blight infection pressures and Phytophthora efficiency (pew-values) were computed by the SIMPHYT3 model to assess conduciveness of climatic conditions for disease development. Simulation results (recommended fungicide treatment) of SIMPHYT1 model predicted first application dates of July 11, 21, 8, 10, 7 and 7 for 2004 to 2009, and for the modified SIMPHYT1 model (US-version) on July 11, 22, 8, 19, 7, and 7 for the same years. Comparison of simulation results with date of disease outbreak in untreated plots resulted in differences of 24–65 days. Validation of the models (differences between recommended fungicide treatment and first blight outbreak) gave better fit for models with predicted intervals of 6–20 days from initial fungicide application to first late blight outbreak. The SIMBLIGHT1, SIMPHYT1, and NOBLIGHT models were accurate and flexible in forecasting the timing of first fungicide applications for disease control. Due to the conducive conditions for late blight potential and infection pressures, development of predictive models that can account for external inoculum sources will greatly improve late blight management at regional or national scales.

Insights into sexual reproduction in *Aspergillus flavus* from variation in experimental crosses and natural populations

R. A. OLARTE (1), B. W. Horn (2), J. T. Monacell (3), E. A. Stone (4), I. Carbone (1)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA, U.S.A.; (3) Bioinformatics Research Center, North Carolina State University, Raleigh, NC, U.S.A.; (4) Department of Genetics, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S92

Aspergillus flavus contaminates many important crops worldwide and is the major producer of aflatoxins, which are cancer-causing secondary metabolites. Biological control is the most effective means of reducing inoculum levels of detrimental aflatoxin-producing fungal pathogens in agricultural systems; however, the long-term efficacy of such methods may face scrutiny with the recent discovery of the sexual cycle in these fungi. We crossed strains of opposite mating type in *A. flavus* to produce offspring, which were genetically and phenotypically analyzed to quantify gene flow and determine the heritability of aflatoxin (AF) and cyclopiazonic acid (CPA). We found that a single generation of sexual reproduction between a nonaflatoxigenic parent containing a single mutation in the aflatoxin cluster and an aflatoxigenic parent can restore aflatoxin production. The recombinant F1 progeny regained aflatoxigenicity through a crossover event within the aflatoxin gene cluster. Other F1 progeny in crosses between either a partial aflatoxin cluster strain or a strain missing the entire cluster and an aflatoxigenic parent regained toxicity via independent assortment of chromosomes. We also found that genetic exchange and recombination are associated with increased heritability of AF and CPA in progeny. These results suggest that a single round of sexual reproduction in *A. flavus* can generate contemporary patterns of recombination and toxin diversity.

Weather patterns and the distribution of Asian soybean rust in the United States

R. O. OLATINWO (1), R. C. Kemerait (2), J. O. Paz (3), G. Hoogenboom (1)
(1) University of Georgia, Griffin, GA, U.S.A.; (2) University of Georgia, Tifton, GA, U.S.A.; (3) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S92

Asian soybean rust (ASR) caused by the fungus *Phakopsora pachyrhizi*, was reported for the first time in the United States in late 2004. The disease is mostly restricted to the south, although it has shown a gradual northward movement during the last few years. The fungus prefers moderate temperatures and wet environments. The objective of this study was to determine the effects of precipitation and temperature on the distribution of ASR incidence in the U.S. Confirmed cases of ASR incidence on kudzu and soybean from 20 states was evaluated using data from the Integrated Pest Management Pest Information Platform for Extension and Education (IPM PIPE), and weather data from the National Climate Data Center (NOAA-NCDC). The initial results showed a significant upward trend in the percentage of counties with ASR infection from 2005 to 2009 in most states including Alabama, Georgia, Mississippi, and Louisiana. When the average statewide monthly temperature in June was ≤24.2°C only 6% of the overall counties had ASR infection, while approximately 36% of counties had ASR infection when the June temperature was higher. When the average statewide June temperature was >24.2°C and the average statewide September precipitation was ≥155.4 mm, the percentage of counties with ASR increased to 69%. Above normal statewide precipitation increased the percentage of counties with ASR infection. Accurate information about favorable conditions for ASR infection could help growers make informed management decisions.

Difenoconazole baseline sensitivity distribution of *Colletotrichum coccodes* isolates from potatoes

G. OLAYA (1), A. Cochran (2), G. Neil (3)
(1) Syngenta Crop Protection, Vero Beach, FL, U.S.A.; (2) Syngenta Crop Protection, Greensboro, NC, U.S.A.; (3) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S93

The sensitivity to the demethylation inhibitor fungicide difenoconazole of 65 monoconidial isolates of *Colletotrichum coccodes* was determined under *in-vitro* conditions. *C. coccodes* solates were collected from potatoes infected with black dot disease that had not been previously exposed to difenoconazole. The geographical origin of the isolates is broad and included several potato growing regions in different States. Sensitivity of each isolate was determined by comparing the colony radial growth on ½ strength potato dextrose agar plates either amended or not with difenoconazole. The sensitivity distributions (ED50 values) of *C. coccodes* isolates ranged from 0.034 to 0.167 with a mean value of 0.065 mg/L. Difenoconazole baseline information will help in future difenoconazole sensitivity monitoring studies looking for the early detection of changes in sensitivity of *C. coccodes* to difenoconazole. Difenoconazole is being developed as a postharvest fungicide for stored potatoes to control diseases caused *C. coccodes*, *Fusarium* spp. and *Helminthosporium solani*.

Fludioxonil sensitivity monitoring of *Penicillium expansum* isolates collected from apples in Washington State

G. OLAYA (1), A. Mulcahy (1), A. Cochran (2)
(1) Syngenta Crop Protection, Vero Beach, FL, U.S.A.; (2) Syngenta Crop Protection, Greensboro, NC, U.S.A.
Phytopathology 100:S93

The sensitivity to the fungicide fludioxonil (commercial name Scholar®) of 103 monoconidial *Penicillium expansum* isolates collected from packing houses where the fungicide have been used was determined under in-vitro conditions. The objective of the fludioxonil monitoring was to determine if changes in sensitivity to fludioxonil were occurring in *P. expansum* isolates. *P. expansum* isolates were collected in 2009 from 5 different apple varieties from commercial packing houses in Washington State. The distribution of fludioxonil sensitivities of 103 *P. expansum* isolates collected in 2009 was similar to the fludioxonil baseline sensitivity distribution that was established in 2004 before the commercial introduction of Scholar®. Sensitivities of isolates collected in 2009 ranged from 0.001 to 0.326 with a mean ED50 value of 0.033 mg/L, whereas the sensitivities of base-line isolates tested in 2004 ranged from 0.006 to 0.171 with a mean of 0.028 mg/L. In conclusion, no important changes in the sensitivity to fludioxonil were detected in *P. expansum* isolates collected in 2009 in Washington State.

Development of a high throughput and fast system for testing transgenic resistance constructs derived from *Grapevine fanleaf virus*

J. E. OLIVER (1), M. Fuchs (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 100:S93

Grapevine fanleaf virus (GFLV) from the genus *Nepovirus*, family *Secoviridae* is transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index* and causes fanleaf degeneration disease. This disease is widespread throughout the world nearly everywhere grapes are grown and the nematode vector is found. Management of GFLV has largely relied on control of its nematode vector, essentially through soil disinfection and the use of resistant rootstocks, although resistance to *X. index* does not prevent GFLV translocation from rootstocks into scions or transmission in vineyards. Resistance to GFLV has not been identified in wild or cultivated grapes; nonetheless, transgenic grapevine rootstocks expressing the full-length viral coat protein gene can confer resistance to GFLV. Based on recent knowledge concerning the genetic variability of GFLV, it is unlikely that transgenic grapevine rootstocks expressing the full-length coat protein gene will show durable resistance. Therefore, new GFLV constructs potentially capable of providing resistance have been developed. However, due to the extended time and expense involved in the development and testing of transgenic grapes for resistance to GFLV, efforts to develop a high throughput approach for testing candidate constructs in *Nicotiana benthamiana*, a systemic host, have been pursued. Results from the development and evaluation of GFLV-derived genetic constructs for engineered resistance will be discussed.

Races of *Puccinia graminis* f. sp. *tritici* with virulence on *Sr13* and *Sr9e* in durum screening nursery in Ethiopia

P. OLIVERA FIRPO (1), Y. Jin (2), A. Badebo (3), D. Singh (4)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) USDA-ARS Cereal Disease Laboratory and University of Minnesota, St. Paul, MN, U.S.A.; (3) Ethiopian Institute of Agricultural Research, Debre Zeit, ETHIOPIA; (4) International Maize and Wheat Improvement Center (CIMMYT), Nairobi, KENYA
Phytopathology 100:S93

Durum wheat (*Triticum turgidum* ssp. *durum*) of North America has a higher frequency of resistance to race TTKSK (or Ug99) of *Puccinia graminis* f. sp. *tritici* than common wheat based on evaluations conducted in Njoro, Kenya. We postulated TTKSK resistance in durum is likely due to *Sr13*, a common gene in North American cultivars. However, when resistant selections were evaluated in Debre Zeit, Ethiopia, many became susceptible to stem rust, suggesting that local races may possess a virulence combination that overcomes the TTKSK resistance. The objective of this study was to identify and characterize races of *P. graminis* f. sp. *tritici* present in the Debre Zeit screening nursery in 2009. Single-pustule isolates were derived from collected samples and race-typed based on the North American stem rust differentials. The isolates were further characterized on a set of universal resistant lines. Three races of *P. graminis* f. sp. *tritici* were identified: JRCQC, TRTTF and TTKSK. Both JRCQC and TRTTF possess virulence combination on *Sr13* and *Sr9e*, which may explain why the TTKSK-resistant durum in Kenya became susceptible in Debre Zeit. The virulence combination on *Sr9e* and *Sr13* is of big concern because these genes constitute the main components of stem rust resistance in North American durum cultivars. In addition to virulence on *Sr9e* and *Sr13*, race TRTTF appears to be virulent to stem rust resistance conferred by the 1AL.1RS translocation in winter wheat in the United States.

Resistance to race TTKSK of *Puccinia graminis* f. sp. *tritici* in tetraploid wheat

P. OLIVERA FIRPO (1), Y. Jin (2), S. Xu (3), D. Klindworth (3)
(1) University of Minnesota, St. Paul, MN, U.S.A.; (2) USDA-ARS Cereal Disease Laboratory and University of Minnesota, St. Paul, MN, U.S.A.; (3) USDA-ARS, Northern Crop Science Laboratory, Fargo, ND, U.S.A.
Phytopathology 100:S93

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 the adapted cultivars have resistance to these races. In attempts to identify new stem rust resistance genes effective against race TTKSK, we evaluated cultivated tetraploid wheat (*T. turgidum* ssp. *dicoccum*, ssp. *carthlicum*, ssp. *polonicum*, ssp. *turanicum*, and ssp. *turgidum*) for resistance to TTKSK and other races with broad virulence. A high frequency of TTKSK resistance at the seedling stage was observed, as 207 (21% of 1002 accessions) exhibited low infection types. Low infection types ranging from 2= to 2+ to race TTKSK were predominant. 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. Fifty accessions were evaluated for resistance to TTKSK and Ethiopian races in the field screening nursery at the Ethiopian Institute for Agricultural Research (Debre Zeit, Ethiopia). Twenty-three accessions exhibited resistant to moderately resistant responses to stem rust. Four accessions susceptible to TTKSK at the seeding stage were resistant at the adult stage. These accessions may possess adult plant resistance. Since all these tetraploid species share the same genome as durum wheat and are in cultivated form, resistance genes could be easily transferred to durum wheat by conventional breeding approaches.

Phylogenetic history and genetic diversity of *Phytophthora cryptogea* and *P. drechsleri* isolates from floriculture crops in North Carolina greenhouses

H. A. OLSON (1), I. Carbone (1), M. Benson (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S94

Since *P. cryptogea* was described in 1919 and *P. drechsleri* 12 years later, one of the most challenging taxonomic situations in the genus is discerning these species. Using isolates collected from NC floriculture crops, the evolutionary history and genetic diversity of *P. cryptogea* and *P. drechsleri* were explored. Initially, 66 isolates representing 13 location-host groups were sequenced at multiple loci. Sequences of all isolates within a group were identical at all loci, so a subset of isolates were selected, cloned to resolve heterozygous sites, and subjected to analysis using SNAP Workbench. The internal transcribed spacer region and cytochrome oxidase II genealogies were congruent and indicated that *P. cryptogea* and *P. drechsleri* are well-supported sister species diverged from a common ancestor with no evidence of gene flow. At both loci, *P. cryptogea* is more genetically diverse than *P. drechsleri*. In contrast, evidence of recombination between *P. cryptogea* and *P. drechsleri* isolates was found in the beta-tubulin (btub) locus, suggesting gene flow between species. Coalescent analysis based on a non-recombining partition in btub showed an initial (older) split between *P. cryptogea* and *P. drechsleri* with a later (recent) event separating the remaining *P. cryptogea* haplotypes from *P. drechsleri*. This may indicate recent gene flow between species or that *P. cryptogea* is polyphyletic and some lineages of *P. cryptogea* share a recent common ancestor with *P. drechsleri*.

Characterization of the MADS-box family of transcription factors in *Fusarium verticillioides*

C. ORTIZ (1), W. Shim (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S94

Transcription factors (TFs) are protein complexes that activate or repress transcription of genes by specifically binding to target recognition sites. MADS-box TFs are a superfamily of regulators that bind to the regulatory motif CArG-box of functionally diverse target genes. This family is well studied in plants, but little is known in fungi. MADS-box TFs have been characterized in select ascomycetes and have been shown to play a role in pathogenicity, cell and fruiting body development, and mating. Our research aim is to elucidate the function of two MADS-box TFs in *Fusarium verticillioides*, a filamentous fungus with worldwide distribution and direct association with ear and stalk rots of corn. The fungus also produces the mycotoxin fumonisin B1 (FB1) which has been linked to human and animal illnesses. To study these TFs, we generated *MADS1* and *MADS2* gene knock-out mutants via homologous recombination. On V8 agar, *MADS1* mutant produced a purple pigment while *MADS2* did not differ from the wild type. Additionally, when grown on corn, *MADS1* and *MADS2* mutants produced significantly less (<10%) FB1 compared to the wild type progenitor. We are in the process of generating knock-out mutants of these MADS genes in the complementary mating type as well as a double mutant. Sexual and asexual development assays, transcriptome analyses, and pathogenicity tests with these mutants will help us further elucidate the function of MADS-box TFs in *F. verticillioides*.

Oomycete research at undergraduate institutions: An update on *SPACES*, an internet resource for and by the oomycete community

M. D. OSPINA-GIRALDO (1), C. Bentley (1)
(1) Lafayette College, Easton, PA, U.S.A.
Phytopathology 100:S94

Comprehensive information on oomycete research can be found in several web portals, including the Oomycete Molecular Genetics Research Collaboration Network website, which provides an excellent source of up-to-date information on events, meetings, fellowships, and important news for the community. In addition, the databases maintained by the Broad Institute, the Joint Genome Institute, and the Virginia Bioinformatics Institute contain and share a wealth of resources focusing on recently sequenced genomes of several oomycetes. The increasing availability of the Internet has made these portals easily accessible to most members of the community. A complementary resource would be one that facilitates member interaction and becomes a forum that makes the exchange of ideas possible. The Oomycete Undergraduate Molecular Genetics Network (OUMGN) has been developed as a means to fill this need; although originally geared towards members of the oomycete research community affiliated with undergraduate institutions, it also welcomes individuals from major universities and other organizations. The OUMGN *SPACES* site is a place where OMGN members and non-members can post questions, comments, announcements, links of interest, etc. Details on its characteristics, advantages, and use will be presented.

Gene transcription patterns in *Phytophthora infestans* cultures grown *in vitro* and *in planta*

M. D. OSPINA-GIRALDO (1), E. Laird (1), C. Mingora (1)
(1) Lafayette College, Easton, PA, U.S.A.
Phytopathology 100:S94

A total of 49 putative genes homologous to members of 8 families belonging to the Carbohydrate esterase (CE) gene superfamily have been identified in the genome of the oomycete *Phytophthora infestans*. It has been suggested that CE enzymes, such as the ones classified within the CE Family 5 (the "cutinase" family) may play a role in the infection process by targeting and degrading the cell wall. Because no EST evidence supporting the expression of some of these genes was available, we analyzed the expression of a subgroup of CE-coding genes using reverse-transcription PCR (RT-PCR) and found that most genes are expressed in mycelium of *P. infestans* grown in vitro. To determine the level of expression of each of these genes, a quantitative PCR (qPCR) analysis was conducted. In addition, because of the potential importance of cutinase for *P. infestans* pathogenicity, a qPCR study was performed using plant tissue samples obtained at different stages of the infection process. Results of these investigations will be presented and discussed.

Foliar chlorophyll content of ponderosa pine on black stain root disease sites after prescribed burning and subsoiling treatment combinations

W. J. OTROSINA (1), P. C. Spaine (1), S. S. Sung (2), W. Woodruff (3), J. T. Kliejunas (4)
(1) USDA Forest Service, Athens, GA, U.S.A.; (2) USDA Forest Service, Pineville, LA, U.S.A.; (3) USDA Forest Service, Susanville, CA, U.S.A.; (4) USDA Forest Service, Kent, WA, U.S.A.
Phytopathology 100:S94

A long term study involving underburning, subsoiling and subsoiling X underburning treatments along with untreated control plots were initiated in 2000 in the Lassen National Forest, California. The study site has active blackstain root disease. A tree within each of three randomly selected grid points among nine located within treatment plots was selected for foliar sampling. Each treatment was replicated four times in a randomized complete block design. Two branches per tree were obtained at mid crown from opposite sides of sampled trees by shooting with a 12 ga shotgun. Harvested needles were placed immediately in an ice chest and later analyzed for chlorophyll a and b content. Six years of needles were commonly retained, thus a short history of chlorophyll status is obtained for this species. Overall, needles from severely symptomatic trees (based on ground observations of gross needle color and appearance) had about 40% of fresh weight and needle length of non symptomatic or slightly symptomatic trees. Total chlorophyll content also declined with decline in needle fresh weight. Prescribed burn treatments also affected needle retention, with the burn treatment having the lowest average needle retention (25% for 2004) compared to the control (63% for 2004), indicating a long term treatment effect.

Fairy ring disease of cranberry: Dissecting the life cycle and development of control strategies

P. V. OUDEMANS (1), J. Polashock (2), J. Vaiciunas (1)
(1) Rutgers University, Chatsworth, NJ, U.S.A.; (2) USDA ARS, Chatsworth, NJ, U.S.A.
Phytopathology 100:S94

Fairy ring is a serious disease affecting cultivated cranberries in New Jersey and Massachusetts. The disease is expressed as expanding patches of dead vines leading to long term yield loss. Recently, we observed dark infection pads associated with dying vines and subsequently isolated the pathogen and confirmed pathogenicity. Sequence analysis showed the causal agent to be a species of *Helicobasidium* (teleomorph) and the anamorph isolated from cranberry was identified as *Thanatophytum* sp. A second anamorphic phase of this pathogen, *Tuberculina*, a rust mycoparasite, may function as the recombinant stage of the pathogen life cycle. A collection of isolates from New Jersey and Massachusetts revealed a high level of diversity as determined by vegetative compatibility (VC) and RAPD analyses. Isolates collected from individual rings were identical whereas most isolates collected from different rings were distinct. This suggests that an active sexual phase is responsible for dissemination. The rust myco-parasite stage may be an important component of the fairy ring life cycle. In 2009, we found the Tuberculina stage on a rust infecting greenbrier (Smilax sp.), a common weed in cranberry beds. These isolates were identical to the cranberry pathogen based on ITS sequence. A complex life cycle has emerged and includes at least three distinct stages on distinct host species. Control of the briar rust may limit spread of the disease into cultivated cranberry beds.

Challenges and constraints impacting development of new and novel plant disease management solutions

D. G. OUIMETTE (1)
(1) Dow AgroSciences LLC, Indianapolis, IN, U.S.A.
Phytopathology 100:S95

Significant market driving forces and constraints with current plant disease management technology requires the continued development of new solutions (such as fungicides) to ensure the viability of agricultural crop production. Such driving forces include: 1) biological (fungicide resistance); 2) regulatory; and 3) public/food chain perceptions of food safety in crops treated with fungicides The development and registration of new fungicides require extensive field characterization to fully understand the strengths and limitations of the new technology, and this process can take up to ten years from initial discovery to final product launch. Due to the complex nature of the product characterization process, various constraints must be effectively managed. These constraints include the following: suitable trial location and environmental manipulation to ensure sufficiently high disease development; reducing the impact of unwanted pests (in particular insects and non-target diseases); government regulations requiring permits for chemical and biological shipment; and finally technical expertise among field scientists to implement successful trials. Global characterization strategies require leveraging both the southern and northern hemisphere for trial locations to take advantage of unique marketplace and environmental conditions and for the ability to conduct research trials throughout the year. The effect of these constraints on efficient product characterization will be discussed.

Microarray analysis of tomato gene expression reveals complex effects on hormone signaling associated with viroid infection

R. OWENS (1), K. Tech (1), J. Shao (1), N. Mock (1), T. Sano (2)
(1) USDA ARS, Beltsville, MD, U.S.A.; (2) Hirosaki University, Hirosaki, JAPAN
Phytopathology 100:S95

Viroids are small non-coding RNAs that use the plant transcriptional machinery to replicate their genomes. Microarray analysis of tolerant (Moneymaker) and sensitive (Rutgers) tomato cultivars infected with the type strain of *Potato spindle tuber viroid* (PSTVd) revealed significant changes in the expression levels of 8.8% or 24.6% of the >10,000 genes included on the Affymetrix array. Three weeks post inoculation, as epinasty and stunting began to intensify in systemically infected leaf tissue, evidence of a general stress response was detected in infected Rutgers plants. Synthesis of ribosomal proteins (primarily cytoplasmic) and ubiquitin-associated protein turnover increased, and expression of many chloroplast-associated genes was repressed. In addition to the salicylic acid-mediated response pathway activated by both RNA and DNA viruses, an unusually large number of genes associated with abscisic acid and brassinosterioid signaling were also affected by PSTVd infection. Both gibberellin and brassinosteroid signaling appear to be involved in PSTVd-induced stunting; in each case, these effects may be due to changes in hormone synthesis/degradation as well as signaling per se. The possible role of PSTVd-related small RNAs as a mediator of post-transcriptional gene silencing is under investigation using a deep sequencing strategy.

Development of a Sweet potato leaf curl virus infectious clone for agroinfection

V. PAHALAWATTA (1)
(1) Alcorn State University, Lorman, MS, U.S.A.
Phytopathology 100:S95

Sweet potato (Ipomoea batatas) is an important root crop and source of industrial raw material worldwide. Virus diseases cause significant losses in sweet potato production both in terms of quality and yield. Sweet potato leaf curl virus (SPLCV) is among the six most important sweet potato virus diseases prevalent in the U.S. SPLCV infection in recent years resulted in 25–30 percent yield losses to the cultivar 'Beauregard' that accounts for approximately 80 percent of the U.S. production. SPLCV is a whitefly-transmitted monopartite single-stranded DNA begomovirus. An alternative method is being tested to infect sweet potato plants with SPLCV in the absence of the insect vector. Full length genomic DNA of SPLCV was cloned and sequenced. The restriction digested DNA fragment containing the viral replication origin was ligated into the corresponding restriction sites of the binary vector pBI121 resulting in pBI121-SPLCV-ori. Full length SPLCV genomic DNA was then cloned into pBI121-SPLCV-ori to obtain the tandem repeat dimer construct pBI121-SPLCV. The SPLCV infectious clone will be introduced into sweet potato plants using an Agrobacterium tumefaciens/Ti plasmid-mediated delivery system. Development of an effective inoculation method independent of the whitefly vector will greatly facilitate high-throughput screening of resistant germplasm, identification of resistant genes as well as understanding the molecular basis of SPLCV-host interactions.

Pathogenicity of *Fusarium oxysporum* f. sp. *radicis cucumerinum* and control strategies of cucumber root and stalk rot in hydroponic systems

D. PALMERO (1), M. De Cara (2), M. Santos (2), C. Iglesias (1), J. Tello (2)
(1) Universidad Politecnica de Madrid, Madrid, SPAIN; (2) Universidad de Almería, Almeria, SPAIN
Phytopathology 100:S95

Pathogenicity of 6 isolates of *Fusarium oxysporum* f. sp. *radiciscucumerinum* was studied on four cucurbitaceae species, one pumpkin hybrid (*Cucurbita maxima* X *C. moschata*), and on eight species belonging to different botanical families. All six *F. oxysporum* isolates showed pathogenicity on three cucurbitaceae, but didn't on squash and pumpkin hybrid, and didn't on each of eight other species different to cucurbitaceae. Severity of the disease was slightly higher at 17°C than 25°C. Control strategies against cucumber root and stalk rot were also tested. Disinfection of the substrate (perlite) and use of cucumber plants (cv Borja) grafted on hybrid pumpkin were evaluated to control the disease. For chemical disinfection of the substrate three different products were used: metam potassium, metam sodium and chloropicrin + 1.3 dichloropropene, these treatments were tested both with and without solarization. Results showed that chemical disinfectants and solarization, failed to successfully control the disease during the two usual crop periods in the area (autumn and spring). The protection for some treatments did not reach the 10 months that included the two cultural cycles. On the other side eight commercial rootstocks were evaluated. All the grafted varieties expressed a complete resistance and a significant increase in production was observed. Keywords: Melon, cucumber syndrome, *Cucurbita pepo* x *C. moschata*, *Cucumis sativus*, *Fusarium* disease.

Pathogenicity and fusaric acid production by *Fusarium proliferatum* isolated from garlic in Spain

D. PALMERO LLAMAS (1), M. de Cara (2), W. Nosir (3), C. Iglesias (1), M. García (1), S. Woodward (3), J. Tello (2)
(1) Technical University of Madrid, Madrid, SPAIN; (2) Universidad de Almería, Almeria, SPAIN; (3) University of Aberdeen, Aberdeen, SCOTLAND
Phytopathology 100:S95

Fusarium proliferatum has been reported on garlic in the north west U.S.A., Spain and Serbia, causing as water-soaked tan lesions on cloves. Moreover, *F. proliferatum* is known to produce a range of toxins, including fumonisin B1, moniliformin, beauvericin, fusaproliferin and fusaric acid, which are implicated in pathogenesis. In this study six randomly selected *F. proliferatum* isolates from garlic were tested for pathogenicity and screened for fusaric acid production. Healthy seedlings of onion (*Allium cepa*), leek (*A. porrum*) and chives (*A. schoenoprasum*) and garlic clones (*A. sativum*) were inoculated. Onion seedlings and garlic clones were soaked in the conidial suspensions of each *F. proliferatum* isolate for 24 h and then planted in flats containing soil previously inoculated with the same isolate of *F. proliferatum*. Plants were maintained in a temperature and light-controlled greenhouse (12 h/12 h light/dark; 25/21°C). The root and bulb/clove rot disease symptoms were graded into five classes following the method of Stankovick *et al.* (2007). A disease severity index (DSI) was calculated as the mean of three plants of each species and four test replicates. Symptoms on onion and garlic plants were observed three weeks after inoculation. The overall effects of isolate, host and variety were analyzed. Effects were significant for all the studied isolates. The correlations between isolate pathogenicity and production of FA are also discussed.

Isolation of *Aspergillus* section Nigri strains and incidence of ochratoxin A in California raisins

J. D. PALUMBO (1), T. L. O'Keeffe (1), N. E. Mahoney (1), S. Vasquez (2)
(1) USDA ARS WRRC, Albany, CA, U.S.A.; (2) University of California Coop Ext, Fresno, CA, U.S.A.
Phytopathology 100:S95

Species of *Aspergillus* section Nigri, particularly *A. niger* and *A. carbonarius*, have been implicated as sources of ochratoxin A (OTA) contamination in wine and table grapes, as well as raisins and other dried fruits. OTA contamination of these commodities is not uncommon in Mediterranean and South American regions, but has not been reported in California vineyards. To investigate the occurrence of OTA-producing *Aspergillus* section Nigri species in California, four raisin vineyards were sampled during the 2009 harvest. Thirty seven of the 40 raisin samples contained measurable OTA contamination. From these raisin samples, a total of 400 strains of *Aspergillus* were isolated and analyzed for production of OTA on culture media. Of these, 13 isolates, from six raisin samples, produced OTA. These isolates were identified as *A. carbonarius* (12 isolates) and *A. niger* (1 isolate), based on morphological characteristics and multilocus sequence analysis. *A. carbonarius* was only recently reported as a causal agent of sour rot on table grapes in California. This is the first report of OTA production by *A. carbonarius* or *A. niger* isolated from California raisins.

Dynamics and single nucleotide polymorphisms of rice blast resistance alleles at the *Pik* locus

Q. PAN (1)
(1) South China Agricultural University, Guangzhou, Guangdong, PRC PEOPLES REP OF CHINA
Phytopathology 100:S96

The *Pik* locus located on rice chromosome 11, which consists of *Pik*, *Pik-s*, *Pik-p*, *Pik-m*, and *Pik-h* alleles, is an important resistance gene resource for rice blast breeding programs in China. Dynamics of resistance of these alleles was characterized with more than 10 populations of *Magnaporthe oryzae* collected from various regions in China. Each allele was an independently and dominantly acting gene at the *Pik* locus, and conditions differential reactions against many isolates. For molecular characterization of the alleles, the genetic and physical maps of these alleles, respectively, were constructed using genomic position-ready markers. Then, the alleles were isolated, respectively, through an approach called map-based cloning, *in silico*. The function of each allele was dissected using both forward and reverse genetic approaches. The evolutionary relationships among alleles were determined by the allele-specific single nucleotide polymorphisms (SNPs) using a large range of rice germplasms. The detailed results will be presented in the meeting.

Genotypic and pathotypic diversity of the *Xanthomonas oryzae* pv. *oryzicola* in southern China

R.-W. Pan (1), W.-C. Zou (1), D.-G. XU (1), R.-Q. Pan (1), C.-Y. Ji (1)
(1) Department of Plant Pathology, South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S96

Rice bacterial leaf streak (BLS), caused by the pathogen *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), is currently one of the most important diseases in southern China, and the utilization of resistance is the major measure to control the disease. Total 179 strains of the pathogen were collected from four provinces in southern China, and the pathotype and the population genetic of the pathogen were studied. PCR-based DNA fingerprint and virulence analysis were used to evaluate the genetic diversity and population structure. Three specific primers ERIC, BOX, and J3, each produced 150, 137, and 126 haplotypes, respectively. Combined the data from three primers, at a similarity level of 0.73, 11 clusters were grouped. Cluster D, represented 31.3% of the strains, was the predominant group in southern China. The value of the genetic diversity (0.826) indicated the highly genetic diversity of the pathogen population in southern China. These strains were classified into ten groups on the basis of the phenotypic reaction on six differential rice cultivars (IRBB4, IRBB5, IRBB14, IRBB21, IR24, and Jingang30). Strains obtained from four provinces or different planting season shared various DNA fingerprints. Pathotype I was the predominant group in southern China including 31.28% of the strains. No correlation was observed between DNA fingerprints and pathotypes of the pathogen.

Identification of soybean genes that contributes to *Rpp2*-mediated defense against Asian soybean rust using VIGS

A. K. PANDEY (1), C. Yang (1), C. Zhang (1), K. F. Pedley (2), M. Graham (3), J. H. Hill (1), S. A. Whitham (1)
(1) Plant Pathology, Iowa State University, Ames, IA, U.S.A.; (2) Foreign Disease-Weed Science Research Unit, United States Department of Agriculture-Agricultural Research Service, Ft. Detrick, MD, U.S.A.; (3) Corn Insects and Crop Genetics Research Unit, United States Department of Agriculture-Agricultural Research Service, Ames, IA, U.S.A.
Phytopathology 100:S96

Asian soybean rust (ASR) is an aggressive foliar disease caused by the obligate biotrophic fungus Phakopsora pachyrhizi. On susceptible cultivars of soybean, the disease symptoms are characterized by tan lesions and abundant uredinia. None of the commercially grown elite cultivars of soybean are resistant to all the isolates of P. pachyrhizi. Germplasm screening efforts have identified five different genes that provide varying levels of resistance to specific isolates of P. pachyrhizi (Rpp1 - Rpp5). Resistance in Rpp2 plants (PI230970) is characterized by the formation of reddish-brown (RB) lesions, limited fungal growth, and little or no sporulation. In the present work, we utilized virus-induced gene silencing (VIGS) to identify genes that contribute to Rpp2-mediated resistance. We screened approximately 140 genes and identified eleven that compromise resistance when silenced. Gene silencing was confirmed by semi-quantitative RT-PCR and fungal growth was measured by quantifying P. pachyrhizi α-tubulin transcript in the silenced leaf tissue. A clear correlation was observed between the increase in fungal growth and the silencing of the eleven soybean genes identified, suggesting their involvement in Rpp2-mediated disease resistance Our results provide fundamental information on the signaling networks that contribute to resistance towards ASR, and will assist further efforts to engineer durable resistance.

Identification of *Xanthomonas* sp. using resonance Raman spectroscopy

M. L. Paret (1), S. K. Sharma (2), A. K. Misra (2), T. Acosta (2), A. S. de Silva (1), T. Vowell (1), A. M. ALVAREZ (1)
(1) Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI, U.S.A.; (2) Hawaii Institute of Geophysics and Planetology, Honolulu, HI, U.S.A.
Phytopathology 100:S96

We have used resonance Raman spectroscopy with 514.5 nm laser excitation for characterizing xanthomonadin in numerous plant pathogenic xanthomonads including *Xanthomonas campestris* pvs. *campestris*, *armoraciae*, *abberans*, *citrumelo*, *X. citri*, *X. vesicatoria*, *X. translucens*, *X. axonopodis* pvs. *dieffenbachiae* and *manihotis*. Resonance Raman spectroscopy has been extensively utilized with visible laser excitation in the characterization of carotenoids, which have structurally unique conjugated polyene chain that gives these pigments absorption in the blue-green region of the spectrum. A molecule similar to carotenoids is xanthomonadin, the brominated aryl-polyene pigment in the bacterial genus *Xanthomonas*. Xanthomonadin is a unique marker for the genus, but the identification technique currently used is thin-layer chromatography, which requires a long processing time, and also can have poor resolution. The Raman bands representing the vibrations (ν_1, ν_2, ν_3) of the polyene chain of xanthomonadin is 1004 (ν_3), 1136 (ν_2), 1529 (ν_1), 2267 ($2\nu_2$), and 2656 ($\nu_1+\nu_2$). These separated out from the Raman fingerprints of other bacterial genera, and results were obtained within 5 minutes.

AiiA-mediated quorum-quenching does not affect virulence or toxoflavin experession in *Burkholderia glumae* SL2376

J. PARK (1), S. Han (1), Y. Lee (2), B. B. McSpadden Gardner (3), Y. Kim (1)
(1) Gwangju, SOUTH KOREA; (2) Suwon, SOUTH KOREA; (3) Wooster, OH, U.S.A.
Phytopathology 100:S96

Burkholderia glumae causes bacterial rice grain rot and produces a broad-host range phytotoxin, called toxoflavin, which is a key pathogenicity factor. The quorum sensing signaling pathway and its connection with pathogenicity of *B. glumae* have yet to be clearly elucidated. In an effort to investigate the effects of quorum-quenching on the pathogenicity of *B. glumae*, the N-acyl-homoserine lactonase (*aiiA*) gene from *Bacillus* sp. 240B1 was expressed in *B. glumae* under the control of a constitutive promoter. Production of acyl homoserine lactones in the *aiiA*-transformants was significantly reduced, but no evidence of loss of virulence was observed in terms of causing rice seedling rot and rice grain rot. The *aiiA*-expressing strains displayed wild-type levels of transcription from the genes in the toxoflavin biosynthetic operon and toxin production. The *aiiA*-expressed strains swarmed and formed flagella similar to the wild-type strain. However, the *aiiA*-expressing B. glumae strain reduced the severity of soft-rot when co-inoculated with the soft-rot pathogen, *Pectobacterium carotovorum* SCCI, consistent with its lactonase activity. Our results show that the aiiA-mediated quorum-quenching does not affect virulence or toxoflavin production in *B. glumae* SL2376.

Protein profile differences between soybean accessions resistant and susceptible to soybean rust (*Phakopsora pachyrhizi*)

S. PARK (1), Z. Chen (1), M. C. Ganiger (1), A. A. Fortunato (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 100:S96

Asian soybean rust, caused by fungus *Phakopsora pachyrhizi*, was first discovered in the continental U.S. in late 2004 and has the potential to cause severe yield losses due to that all U.S. commercial soybean varieties are susceptible. In this study, thirteen accessions were evaluated with rust spores collected in Louisiana. Two accessions (PI417089A and PI567104B) showed consistent immune response in both detached leaf assay and greenhouse inoculation. Fungal biomass as determined using qRT-PCR increased significantly 2 days after infection in susceptible lines whereas no or little increase was detected in resistant lines. Protein profiles of these two resistant and two susceptible lines (PI548631 and 93M60) were compared to understand compatible and incompatible host-pathogen interactions at the molecular level using proteomics. Differentially expressed proteins were observed in both resistant and susceptible lines with and without infection. Nine and 16 proteins were identified as induced spots at 1 day after infection in both resistant accessions after comparing to PI548631 and 93M60 susceptible line, respectively. Sixteen spots were sequenced and they belong to plant defense, signaling, and photosynthesis. The transcript levels of differentially expressed proteins were also determined using qRT-PCR.

Distinct roles of two 9-lipoxygenase paralogs in the regulation of aflatoxin accumulation in maize seed

Y. PARK (1), M. Kolomiets (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S96

Colonization of kernels by *Aspergillus flavus* is one of the major limiting factors for maize production worldwide because they contaminate seed with highly carcinogenic aflatoxins. Host and pathogen-derived oxygenated lipids are implicated as signals in the regulation of biosynthesis of aflatoxins. In this study, we tested whether disruption of the host lipoxygenases, *ZmLOX4* and *ZmLOX5*, have an effect on aflatoxin accumulation of seed. The two genes are highly related to each other sharing >94% nucleotide and amino acid sequence identity. Field-based testing for the mutants and near-isogenic wild types showed that *lox5* mutants accumulated significantly lower levels of aflatoxin, whereas *lox4* mutants did not show any significant difference. Northern blot analyses demonstrated differential induction of the two genes in response to infection of silks and kernels with *A. flavus*. Taken together, these data indicate that despite extremely high sequence similarity between the two paralogs, only *ZmLOX5* is required for normal production of aflatoxins under the filed conditions. Potential mechanisms of *ZmLOX5* involvement in the regulation of aflatoxin production are proposed.

Genetic stability of *Magnaporthe oryzae* isolates on the host and artificial media

S. PARK (1), M. Chi (1), H. Kim (1), S. Kang (2), Y. Lee (1)
(1) Seoul National University, Seoul, KOREA; (2) Pennsylvania State University, State College, PA, U.S.A.
Phytopathology 100:S97

Although genetic instability in the rice blast fungus is a highly controversial subject, only a few strains have been used in genetic studies for more than two decades. Here we report genetic stability of *Magnaporthe oryzae* isolates on the host and artificial media. A total of 176 strains were obtained by culturing single spores on artificial medium during successive round of asexual reproduction and by infecting rice plants up to the 10th generation from isolate 70-15. Another 20 strains were recovered from both ends of germ tubes at basal and apical cells from 10 conidia which are generally three-cells. Additionally, 60 strains were recovered by serial transfer on rice plants and the medium from isolate KJ201. All strains exhibited no apparent difference in several phenotypes, including mycelial growth, conidial morphologies, conidial germination, appressorium formation, and pathogenicity, and in DNA fingerprints using MGR586, MAGGY, Line, and MG-SINE as probes. Our data strongly suggest that phenotype and genotype of *M. oryzae* isolates are maintained stably, at least, up to the 10th successive generations on the cultural medium and the host plant.

Phylogenetic and functional characterization of putative forkhead transcription factors in the rice blast fungus

J. PARK (1), J. Jeon (2), S. Park (1), S. Kong (1), J. Park (1), Y. Lee (1)
(1) Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul, SOUTH KOREA; (2) Department of Agricultural Biotechnology, Seoul National University, Seoul, SOUTH KOREA
Phytopathology 100:S97

Forkhead-box protein, named from the two spiked-head mutants of *Drosophila*, is a transcription factor (TF) playing essential roles in a wide range of biological processes such as cell cycle progression, growth regulation, and developmental differentiation in eukaryotic organisms. Although a few forkhead TFs were characterized in yeasts, very little knowledge is available for filamentous fungi. To examine the phylogenetic relationships, a total of 62 putative forkhead TFs, including 4 of *Magnaporthe oryzae*, from 16 fungal species were identified and analyzed. This analysis showed that 2 of *M. oryzae* forkhead TFs (named as MoFKH1 and MoHCM1) are yeast-related and others (MoFOX1 and MoFOX2) are filamentous fungi-specific. Deletion mutants of *MoFOX1*, a filamentous fungi-specific forkhead, were indistinguishable to the wild-type in their phenotypes. No deletion mutant of *MoFOX2* was obtained notwithstanding several attempts of transformation and extensive screening of transformants, indicating the possibility of an essential gene. However, deletion mutants of both *MoFKH1* and *MoHCM1* exhibited pleiotrophic phenotype defects, such as conidial germination, appressoria formation, mycelial growth, and pathogenicity. Furthermore, defects in septa formation were only observed in deletion mutant of *MoFKH1*, but not in *MoHCM1*. These results suggested that yeast-related forkhead TFs in *M. oryzae* showed functional links to the corresponding TFs of *Saccharomyces cerevisiae*.

FEDB: The integrated platform for expressed sequence tags in fungal kingdom

J. PARK (1), S. Kim (2), W. Song (3), S. Kim (3), S. Jang (3), K. Cheong (1), D. Kim (1), J. Choi (1), K. Ahn (3), B. Park (4), D. Choi (5), S. Kang (4), Y. Lee (1)
(1) Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, Center for Fungal Pathogenesis, and Department of Agricultural Biotechnology, Seoul National University, Seoul, KOREA; (2) Fungal Bioinformatics Laboratory, Department of Plant Science, and Department of Agricultural Biotechnology, Seoul National University, Seoul, KOREA; (3) Fungal Bioinformatics Laboratory, Seoul National University, Seoul, KOREA; (4) Department of Plant Pathology, The Pennsylvania State University, University Park, PA, U.S.A.; (5) Deaprtment of Plant Science, Seoul National University, Seoul, KOREA
Phytopathology 100:S97

Regulation of gene expressions plays an essential role in modulating diverse biological processes. One conventional way to survey the expression level is expressed sequence tags (ESTs) which provide messenger RNA sequences under certain conditions. With the combination of new genome sequencing technologies and many fungal genome projects, large amount of fungal ESTs have been generated; however, there is no proper data warehouse for these ESTs. Fungal Expression Database (FEDB; http://fedb.snu.ac.kr/) standardized fungal ESTs with Comparative Fungal Genomics Platform (CFGP; http://cfgp.snu.ac.kr/), allowing free-data exchange with various bioinformatics databases. Currently FEDB archives 3.1 million ESTs from 101 fungal species. To generate unigenes, three-step pipeline was constructed: i) crossmatch was integrated for removing vector sequences, ii) polyA/T filter for eliminating poly A/T sequences, and iii) CAP3 and Phrap for generating unigenes. To provide user-interactive web interfaces, i) Taxonomy Browser to browse ESTs along with fungal taxonomy, ii) SNU Genome Browser (SNUGB; http://genomebrowser.snu.ac.kr/) to present ESTs matched to proper fungal genomes, and iii) Favorite, the personalized virtual space containing various EST analysis tools, were integrated. With the tools implemented in FEDB and the free-data exchange model, FEDB will provide the environment for diverse in-depth analyses of fungal transcriptomics.

Differentiation of *Xylella fastidiosa* strains via analysis of environmentally-mediated genes

J. K. PARKER (1), L. De La Fuente (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S97

Attempts to elucidate the genetic relationships between strains of *Xylella fastidiosa* (XF) have shown that genotypes tend to cluster into groups based on the host plant species from which they were isolated. Comparative genetic analyses have been conducted for isolates from host plants of economic importance including grapes, citrus, and almonds. However, differentiation of strains from within host types (e.g. between grape isolates) and characterization of strains from newly identified hosts (e.g. blueberries) have been limited and/or inconclusive. Here, sequence analysis of environmentally-mediated genes (genes thought to be influenced by environmental factors) was applied to identify strain relationships. Multi-locus sequence analysis (MLSA) was used for genes related to processes important for establishing XF infections such as surface attachment, biofilm formation, virulence, and nutrient transport and utilization. These types of genes may be more relevant to host-based genetic variability. Genes of interest were identified from previous studies and PCR primers were designed from the available whole genome sequences of XF. Target genes were PCR-amplified and sequenced from XF strains isolated from a variety of host plants from several geographic regions in the U.S. Phylogenetic analyses of the resulting sequence information show host-based and geographic origin-based genetic relationships which provide new insights into the epidemiology of populations of XF.

Diversity of the tobacco black shank pathogen, *Phytophthora nicotianae*, in Virginia

V. PARKUNAN (1), C. Johnson (2), C. Hong (1)
(1) VPI & State University, Virginia Beach, VA, U.S.A.; (2) VPI & State University, Blackstone, VA, U.S.A.
Phytopathology 100:S97

Black shank, caused by *Phytophthora nicotianae*, is a significant problem in tobacco-growing regions throughout the world, including Virginia. A total of 217 isolates of *P. nicotianae* were collected from stem pith samples from flue-cured, burley, and dark fire-cured tobacco sampled from fields in 12 Virginia counties in 2006–09. Isolate race identities were determined using host differential assays. Seventy-six percent of the isolates were race 1, 21% race 0, and 3% race 3. The mating type was determined based on a conventional pairing assay using *P. meadii* and/or *P. nicotianae* testers. In contrast to an earlier North Carolina study, 94 percent of the isolates were of the A^2 mating type; only 6 percent of the isolates belonged to the A^1 mating type. A single mating type in most of Virginia's tobacco fields may indicate a lower possibility for sexual recombination, possibly creating a biological bottleneck to adaptation by the pathogen population. A detailed genetic diversity study is underway employing simple sequence repeats and random amplified polymorphic DNA markers.

New races of the rust pathogen in the United States affect *Ur-3* a broadly used rust resistance gene in common bean
M. A. PASTOR-CORRALES (1)
(1) USDA ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S98

Common bean rust is caused by Uromyces appendiculatus, a hyper variable pathogen that is notorious for its capacity to recurrently produce new virulent strains. Rust symptoms were found on dry bean varieties with Ur-3, a gene that had previously conferred resistance to bean rust and had been used extensively in the development of dry bean cultivars. Two new strains of this pathogen were found in Michigan and North Dakota in 2007 and 2008, respectively. These two states are the largest producers of dry beans in the U.S. Our virulence studies revealed two similar but not identical races; one from Michigan (22-2) and the other from North Dakota (20-3). Both races infected the differential cultivars Aurora (Ur-3) and Golden Gate Wax (Ur-6). Neither race infected Early Gallatin (Ur-4), Mexico 309 (Ur-5), Pompadour Checa 50 (U-9, Ur-12), and PI 181996 (Ur-11). These races differed in their virulence; only 22-2 infected Redlands Pioneer (Ur-13) and only 20-3 infected Great Northern 1140 (Ur-7). Other sources of resistance, such as CNC and PI 260418, with uncharacterized rust resistance genes, were also resistant to both new races. Approximately 40 U.S. common bean cultivars were also inoculated with the two new races. The results revealed new dry bean cultivars, some with two or more rust resistance genes possessed a broad rust resistance to the new races from Michigan and North Dakota and to different races from other parts of the world.

Differential reactions of sources of Rpp-resistance to an Rpp-virulent isolate of *Puccinia polysora*
J. K. PATAKY (1), W. E. Dolezal (2), J. L. Brewbaker (3)
(1) University of Illinois, Dept. Crop Sciences, Urbana, IL, U.S.A.; (2) Pioneer Hi-Bred International, Inc., Johnston, IA, U.S.A.; (3) University of Hawaii-Manoa, Dept. Tropical Plant and Soil Sciences, Manoa, HI, U.S.A.
Phytopathology 100:S98

Southern rust, caused by *Puccinia polysora*, occurs on maize grown in subtropical or tropical regions and in temperate climates of the continental United States. It is distinct from common and tropical rusts, caused by *P. sorghi* and *Physopella zeae*. A single dominant gene *Rpp9*, described from PI 186208 (Boesman yellow flint), is used in North America as a source of a chlorotic fleck, resistant reaction against *P. polysora*; however, this gene is ineffective in many parts of the world due to virulent isolates. The *Rpp9* gene is located on chromosome 10S about 1.6 cM from the Rp1 region of genes conveying resistance to *P. sorghi*. Several additional sources of Rpp-resistance map to the same region of 10S as *Rpp9* or they are allelic with *Rpp9* based on resistance of testcross progeny. In 2008, isolates of *P. polysora* virulent on corn lines with the *Rpp9* gene, including PI 186208, were collected in Georgia. Several sources of resistance thought to carry the *Rpp9* gene were resistant when inoculated with a wild-type isolate of *P. polysora* from Illinois, susceptible when subjected to wild type isolates at Waimanalo, Hawaii, but had differential reactions when inoculated with Rpp-virulent isolates from Georgia. The differential reactions of these lines suggests that genes in addition to *Rpp9* are involved in the southern rust resistance of some of these Rpp-resistant lines and/or that the *Rpp9* gene is a complex region of multiple resistance genes similar to the Rp1 region for common rust resistance.

Characterization of Type IV pilus in the bacterial biocontrol agent *Lysobacter enzymogenes* strain C3
N. PATEL (1), B. Hillman (1), D. Kobayashi (1)
(1) Rutgers The State University of New Jersey, New Brunswick, NJ, U.S.A.
Phytopathology 100:S98

Gliding motility and pathogenesis of lower eukaryotes are distinct features of the Gram negative bacterium *Lysobacter enzymogenes*. While both are predicted to play important roles in microbial antagonism by the biological control agent *L. enzymogenes* strain C3, the molecular mechanisms that contribute to these traits are poorly understood. Type IV pili (T4P) are known to contribute to a variety of roles in bacteria, including gliding motility, host adherence and pathogenesis. Genes encoding for the biogenesis of a type IV pilus (T4P) have been identified in three unlinked pil loci within the genome sequence *L. enzymogenes* strain C3. To evaluate the role of T4P in various functions in *L. enzymogenes* C3, a deletion mutation within the major pilin subunit pilA gene was constructed. Colonies of the resultant mutant strain appeared as dry circular colonies with lobate margins during growth on 10% tryptic soy agar, in contrast to fluid circular colonies with entire margins produced by the wildtype strain. The mutant strain also lacked the spreading phenotype of gliding motility on solid growth media. During interactions with fungal hosts, the pilA mutant strain was capable of infecting fungal cells intracellularly in similar manner to the wildtype strain, but it remains unclear whether virulence of the mutant is reduced.

Probability modeling of pecan scab using weather variables as inputs
A. F. PAYNE (1), D. L. Smith (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S98

Pecan scab, caused by the fungus *Cladosporium caryigenum* (syn. *Fusicladium effusum*), affects foliage and fruit of pecan trees resulting in reduced quality and yield. Improving the accuracy of models that predict scab infection would be useful for timing fungicide sprays. Visual ratings of fruit scab severity were collected in 1994, 1995, 1996, and 2009 growing seasons. Daily weather data were compiled from the Oklahoma Mesonet. Moving averages were calculated with respect to days between disease ratings for selected weather variables. Rainfall (≥2.5 mm) and disease severity (≥25%) thresholds were converted to dichotomous variables where 1 was above and 0 below the threshold for each variable. Weather variables were used as independent variables and disease severity (DS) as the dependent variable using generalized estimating equations. Goodness of fit was determined using the quasi-log-likelihood under the independence model information criteria adjusted for number of model parameters (QICu). Minimizing QICu demonstrates better model fit. Weather variables examined were temperature (T), relative humidity (RH), dew point (DP), solar radiation (SR), and total rainfall (R). Model 1 included T, RH, SR, and R to describe DS (QICu = 167.99). Model 2 included DP, SR, and R (QICu = 177.14). The modest increase in QICu of Model 2 demonstrates that DP can be used as accurately as T and RH when modeling DS. These data suggest that DP may be an important predictor of pecan scab.

One Step Construction of Agrobacterium Recombination-ready plasmids (OSCAR), an efficient and robust tool for ATMT gene deletion construction in fungi
Z. Paz (1), S. E. GOLD (1), D. L. Andrews (1), S. J. Klosterman (2), M. D. García-Pedrajas (3), L. Baeza-Montañez (3)
(1) University of Georgia, Athens, GA, U.S.A.; (2) USDA-ARS, Salinas, CA, U.S.A.; (3) Laboratorio de Micologia Estacion Experimental La Mayora, CSIC, Algarrobo-Costa, Malaga, SPAIN
Phytopathology 100:S98

We describe OSCAR, a novel method for the rapid generation of gene deletion constructs for Agrobacterium tumefaciens-mediated transformation (ATMT) in fungi. We designed i) a marker vector with a hygromycin B resistance gene with the Aspergillus nidulans trpC promoter, flanked by the attP1r and attP4 recombination sites and ii) a modified binary vector containing the ccdB gene flanked by attP2r and attP3 recombination sites. 5′ and 3′ gene flanks with attB2r/attB1r and attB4/attB3 recombination sites, respectively are generated by PCR. A single BP clonase reaction containing both the above vectors and PCR flanks results in a deletion plasmid containing the resistance cassette surrounded by the upstream and downstream gene flanks, all contained between the T-DNA borders. The genome sequence of Verticillium dahliae (http://www.broad.mit.edu/) allows functional analysis using OSCAR. Deletion constructs for six genes were obtained at high frequency. One OSCAR construct (VDAG_02161) was tested in fungal ATMT and resulted in a complete gene deletion. Additionally, the method has been validated in several collaborating laboratories and is being used in an undergraduate course at the UGA. In summary, OSCAR combines PCR and Gateway technology, replacing the more time consuming and expensive Invitrogen MultiSite Gateway® Technology, to rapidly and robustly generate precise deletion constructs for ATMT based homologous gene replacement.

Correlation between antibody-binding properties and seasonal disease severity indices of *Dickeya* sp. that cause bacterial heart rot of pineapple
G. D. Peckham (1), A. de Silva (1), J. M. Berestecky (2), A. M. ALVAREZ (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.; (2) Kapiolani Community College, Honolulu, HI, U.S.A.
Phytopathology 100:S98

Bacterial heart rot of pineapple in Hawaii is caused by strains initially identified as *Erwinia chrysanthemi*, now reclassified as *Dickeya* sp. The species designation of pineapple strains awaits full genetic characterization. Based on BOX-PCR the pineapple strains fell into five genetic groups (A-E). Differences in seasonal disease severity were observed between BOX-PCR A and C types, C types being the most severe. Monoclonal antibodies (MAbs) were generated to facilitate rapid detection and identification of these virulent strains from diseased plant material. One antibody (Pine-1, clone 2D11G1; IgG_1 with kappa light chain) recognized all of the strains isolated from Hawaiian pineapple (A-E). Another antibody (Pine-2, clone 2A7F2; IgG_3 with kappa light chain) recognized only a subset of these strains (BOX-PCR types BCD). In the initial outbreak both A and C type strains were frequently isolated. However, in subsequent years only C types have been rediscovered. Since the antibodies distinguish between A and C types, they have value in

on-going studies to evaluate the spread and establishment of the most virulent bacterial populations associated with the initial disease outbreak.

Evaluation of runner bean germplasm for virus resistance

E. Peña-Reyes (1), G. Bascur (1), P. Mendoza (1), P. Sepúlveda (1), I. ROSALES VILLAVICENCIO (1)
(1) Instituto de Investigaciones Agropecuarias, Santiago, CHILE
Phytopathology 100:S99

Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes and the leading source of low cost quality proteins. For several years INIA has developed a bean-breading program whose main objectives are to breed cultivars with special plant habits such as high yields, adaptability to mechanical harvesting and resistance to viral diseases, which are a limiting factor for bean production in Chile. Considering that the most effective way to control viral diseases is the use of resistant material, the main objective of this work was to search for sources of resistance against the most important viral agents affecting this crop in Chile: Bean common mosaic virus (BCMV), Bean yellow mosaic virus (BYMV), Alfalfa mosaic virus (AMV) and Cucumber mosaic virus (CMV). Sixteen accessions of runner bean (*Phaseolus coccineus* L.) were mechanically and naturally infected with each one of the four viruses under study. The plants were regularly evaluated for viral infection by using diagnostic techniques specifically developed for the identification of the pathogens, such as RT-PCR and immunotissue blot. The results indicated that only two accessions were immune to all viruses studied, and 10 of them show resistance to the new and emerging viral complex affecting bean production, which is caused by CMV and AMV. Additionally, we are at this time confirming the first identification of Bean common mosaic necrosis virus (BCMNV) in bean fields located at INIA Research Station.

Management of clubroot (*Plasmodiophora brassicae*) with microbial and synthetic fungicides

G. Peng (1), S. Hwang (2), M. McDonald (3), R. Lahlali (1), B. D. GOSSEN (1), R. H. Hynes (1), S. M. Boyetchko (1)
(1) Agriculture & Agri-Food Canada, Saskatoon, SK, CANADA; (2) Alberta Agriculture and Rural Development, Edmonton, AB, CANADA; (3) University of Guelph, Guelph, ON, CANADA
Phytopathology 100:S99

Clubroot, caused by *Plasmodiophora brassicae*, is an emerging threat to canola (*Brassica napus*) production in western Canada and a serious problem on Brassica vegetable crops worldwide. In inoculated trials on canola under controlled conditions, the biofungicides Serenade® (*Bacillus subtilis*) and Prestop® (*Gliocladium catenulatum*), and the synthetic fungicides fluazinam and cyazofamid reduced clubroot severity substantially when applied as a soil drench at 1.4 Kg, 10 L, 2.9 L, and 0.54 L/ha, respectively. Suspensions of the biofungicides made with pure bacterial or fungal cultures were as efficacious as the formulated products. Cell-free product filtrates were slightly less effective. These fungicides were also evaluated as in-furrow treatment at the same rates as above in three field trials on canola in Alberta and Ontario, and one napa cabbage (*B. rapa* subsp. *Chinensis* var. *utilis*) trial in Ontario. Resistant (R) and susceptible (S) cultivars were assessed in each trial. Plants (20-25 per rep) were assessed for clubroot severity using a 0–3 scale at bloom (canola) or 8 wks after seeding (napa cabbage). Severe spring drought conditions in Alberta delayed germination and reduced seedling establishment. None of the products reduced clubroot on canola in the Alberta trials. For the napa cabbage trial, a rain event occurred 2 d after seeding. The fungicides reduced clubroot severity on the S cultivar by 50–85%. R cultivars reduced severity by 87–93% on canola and 99% on napa cabbage when compared to S cultivars.

Resistance of triploid watermelon cultivars to Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum*

K. PENNERMAN (1), K. Everts (2)
(1) University of Maryland, College Park, MD, U.S.A.; (2) University of Maryland and University of Delaware, College Park, MD, U.S.A.
Phytopathology 100:S99

Yield loss in watermelon due to Fusarium wilt (*Fusarium oxysporum* f. sp. *niveum*) was managed for many years through the use of cultivars that were resistant to *F. o. niveum* race 1. However, the prevalence and severity of Fusarium wilt in watermelon has been increasing in the eastern U.S. in the past decade. This increase has coincided with an increase in production of triploid watermelon cultivars, most of which lack resistance to race 1. A trial was conducted in 2009 in a field at the University of Delaware's Research and Education Center, Georgetown to evaluate thirteen triploid watermelon cultivars that had previously been identified as possessing some level of resistance to one or more races (races 0 and 1) of *F. o. niveum* and a susceptible check cultivar. Watermelons had been continuously planted in the field for eleven years resulting in a moderate level of *F. o. niveum*. 'Matrix', 'Sweet Delight', 'ACX4674T FR', 'ACX5117TSS FR', 'ACR6277TSS FR', 'ACR6177TSS FR', and 'ACX5727 FR' had significantly lower wilt incidence than 'Ruby' and 'Indiana' on 21 July. However, many cultivars did not perform as well as expected. Isolates of *F. o. niveum* were collected and evaluated to determine if *F. o. niveum* race 2 was present in the field. The isolates of *F. o. niveum* along with reference isolates of races 0, 1, and 2 were compared on four differential watermelon genotypes using the root-dip inoculation method. The presence of *F. o. niveum* race 2 was confirmed.

Update on olive pathologies in Argentina

B. A. PEREZ (1), M. L. Otero (2), E. Oriolani (3), M. Roca (4), N. Brancher (5), A. C. Matias (6)
(1) IMYZA-INTA, Hurlingham, Buenos Aires, ARGENTINA; (2) INTA-IFFIVE, Cordoba, ARGENTINA; (3) INTA-EEA, Mendoza, ARGENTINA; (4) SENASA, La Rioja, ARGENTINA; (5) Ministerio de la Produccion, Catamarca, ARGENTINA; (6) INTA EEA, Catamarca, ARGENTINA
Phytopathology 100:S99

Olive (*Olea europaea*) is grown in Catamarca, La Rioja, Mendoza, San Juan, south Buenos Aires, northwest Cordoba, and eastern Rio Negro provinces. The aim was to determine the current health status in the traditional and new olive growing area throughout 2009. Disease surveys included several locations of the cited provinces. Plant samples were analyzed by routine laboratory techniques. Olive knot disease was in both young and old plants in Mendoza, San Juan, and south Buenos Aires in orchards with hail damage. Root rot diseases were recorded in field plants in Buenos Aires (Aparicio, Dorrego), Catamarca (Central Valley), La Rioja (Chilecito), Mendoza (Atuel, Lavalle, Lujan de Cuyo, Maipu), Rio Negro (Conesa, Viedma.), and San Juan (Pocito). Sclerotium basal rot was detected in nursery plants in Catamarca, La Rioja, and San Juan. Plants with Verticillium wilt were present in Chilecito, Lavalle, La Rioja Capital, Lujan de Cuyo, and Maipu. Phomopsis twig blight was noted in field plants (Central Valley). Foliar and fruit diseases were more evident in older orchards and less in younger plants throughout the surveyed olive zone. Partially dry plants were more evident in La Rioja but were also observed in Mendoza, and San Juan. Crown gall is restricted to north and northwest Cordoba. Symptoms such as bleeding in branches and trunks, vitiligo, lumps and lattices appeared in young and old plants in La Rioja (Aimogasta, La Rioja Capital) and Catamarca (Central Valley, Tinogasta). Differente leaf distortions (sickle, bifida, bell and cup shaped leaves) were widespread in the olive zone.

Blueberry pathologies in nursery plants

B. A. Perez (1), E. R. WRIGHT (2), M. Divo de Sesar (2)
(1) IMYZA-INTA, Hurlingham, Buenos Aires, ARGENTINA; (2) Facultad de Agronomía, Universidad de Buenos Aires, Ciudad de Buenos Aires, ARGENTINA
Phytopathology 100:S99

Blueberry plants (*Vaccinium corymbosum*) are obtained by vegetative propagation under different conditions. The purpose of this study was to identify the fungi present in plants raised in nurseries under a black shading net in Buenos Aires. Leaf samples cvs. Sharp Blue and Misty with dark and red spots were collected in summer months (January to March) in 2008 and 2010 and processed in laboratory using routine techniques. A stereo microscope was used to observe rust pustules, to prepare slides for microscopic observations and transfer fungal spores that developed on leaves incubated in moist chambers to 2% WA. Fungal purification was made by subculturing the point of hyphae in WA and then cultured in GYE and MYE agar media. The fungi *Colletotrichum gloeosporioides* and *Pestalotiopsis guepini* were identified. Rust pustules and urediospores of *Pucciniastrum vaccinii* were recorded in the underside of the leaves in correspondence with dark spot on the upper side of affected leaves of cv. Sharp Blue. This cultivar had a susceptible reaction to rust and was also the most susceptible under field conditions in San Pedro area (Buenos Aires).

Alterations of capsid protein amino acid positions internal to virions of Cucumber mosaic virus disrupt nonpersistent virus transmission by aphids

K. PERRY (1), C. Bricault (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S99

In the atomic model of Cucumber mosaic virus (CMV), six amino acid residues form stabilizing salt bridges between subunits of the asymmetric unit. To evaluate the effects of these positions on virion stability and aphid vector transmission, six charged amino acid residues were individually mutated to alanine. Five of the six engineered viruses, mutants D100A, K127A, D176A, D179A, and K182A, were viable and able to systemically infect Nicotiana tabacum and to locally infect Chenopodium quinoa; mutant K101A could only be recovered with difficulty. In order to assess the physical stability of mutants, virions were purified from plants and tested in a urea disruption assay. All five mutant viruses were stable during purification in the presence

of 1.5 M sodium and chloroform and exhibited wild type levels of virion stability in the presence of urea. Aphid vector transmissibility of three of the five mutants, D100A, K127A, and D176A, was nearly eliminated. After a series of mechanical passages, additional second site mutations were selected in four of the five mutant viruses, including in one compensatory mutation that restored wild type levels of aphid transmission. It is hypothesized that non-surface associated amino acids involved in acid-base pairing affect the dynamic properties of virions that are in turn required for aphid vector transmission.

A tospovirus new to North America: Virus detection and discovery through the use of a macroarray for viruses of solanaceous crops
K. PERRY (1), X. Lu (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S100

Winter planted tomato plants with virus-like symptoms of leaf distortion, mottle-mosaic, and necrotic stem streaking were observed in south Florida in January 2010. Extraction of total plant RNAs and analysis using a macroarray for the detection of viruses of solanaceous crops revealed hybridization to 70-mer oligonucleotide probes designed for the detection of tospoviruses. Probes with hybridization signals contained sequences from the genomes of *Tomato chlorotic spot virus* (TCSV), *Impatiens necrotic spot virus*, and *Tomato spotted wilt virus*, with the majority from TCSV. The macroarray did not contain probes for the related *Groundnut ringspot virus* (GRSV), as this virus had not been reported from solanaceous plants. Sequences from the S RNA showed 91% identity with GRSV but only 78% identity with TCSV. Sequencing of an M RNA derived, cDNA fragment suggests a closer relationship with TCSV. A more complete analysis of the viral genome and investigation of its vector relationships in progress will provide a better understanding of the nature of the virus.

Host proteins implicated in the aphid transmission of cereal yellow dwarf virus-RPV
K. PETER (1), M. Cilia (2), F. Gildow (1), T. Thannhauser (2), S. Gray (3)
(1) Penn State University, University Park, PA, U.S.A.; (2) USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY, U.S.A.; (3) USDA-ARS, Robert W. Holley Center for Agriculture and Health and Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Ithaca, NY, U.S.A.
Phytopathology 100:S100

The circulative, persistent transmission of cereal yellow dwarf virus-RPV (CYDV-RPV; Luteoviridae) is orchestrated by interactions between virus and aphid proteins. Here we present evidence that plant proteins are also required for successful virus transmission. Treatment of CYDV–RPV-infected oat tissue homogenate during virus purification with commonly used additives EDTA and sodium sulfite rendered the purified virus non-transmissible by aphids. Virus purified in the absence of additives was aphid transmissible. TEM analysis demonstrated virion structure was similar, and the elecrophoretic migration of the capsid proteins in 1-D SDS PAGE was identical. However, the number and molecular weights of additional proteins consistently co-purifying with virus were different between preparations. Using mass spectrometry, proteins involved in respiration, carbon metabolism and carbohydrate breakdown, and histones were identified that co-purified with both transmissible and non-transmissible virus, indicating these proteins are closely associated with virions during infection, but are not sufficient for aphid transmission. Two key enzymes in fructose metabolism co-purified with only transmissible virus. These results demonstrate that luteovirus transmission is influenced, in part, by host proteins binding to, or closely associating with virions. Additives can affect protein structure and protein-protein interactions and therefore may alter critical interactions necessary for aphid transmission.

A comprehensive management strategy for Sclerotinia rot of carrots
R. D. PETERS (1), K. R. Sanderson (1)
(1) Agriculture & Agri-Food Canada, Charlottetown, PE, CANADA
Phytopathology 100:S100

Sclerotinia rot of carrots (SRC), caused by *Sclerotinia sclerotiorum*, is a devastating disease that causes yield losses in the field and in storage. Research in Prince Edward Island (PEI), Canada on carrots grown in mineral soils has demonstrated that factors that increase canopy density, including higher seeding rates and nitrogen inputs, can significantly increase the severity of SRC. As well, cultivars with a propensity to grow lush canopy architectures are also more susceptible to disease. In an attempt to ameliorate these effects, a prototype carrot foliage trimmer (CFT) was designed and manufactured in PEI in 2006. Several years of field evaluation has indicated that trimming at row closure can significantly ($P = 0.05$) reduce the incidence of SRC on foliage and in carrot roots by about 75%, without compromising carrot yield or quality. Even with these disease reductions, some diseased carrots may still enter storages, where lateral spread of the pathogen from diseased to healthy carrots can occur rapidly. To manage the post-harvest spread of the pathogen, applications of low rates of fludioxonil as a dip treatment as carrots enter storage was shown to virtually eliminate lateral spread of the pathogen in storage crates. Therefore, a comprehensive management strategy for SRC will include careful decisions on cultivar choice, seeding rate and fertility inputs as well as the integration of canopy trimming and post-harvest fludioxonil application.

The relationship between genetic diversity of *Ralstonia solanancearum* and mechanical transmission in tobacco
P. D. PETERSON (1), B. A. Fortnum (1), A. L. Mila (2)
(1) Clemson University, Florence, SC, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S100

Mechanical flower and leaf removal in tobacco have a major role in the spread of bacterial wilt in the southeastern U.S.A. *Ralstonia solanacearum* typically infects tobacco plants through direct penetration of roots, but infection can also occur through topping wounds, leaf scars and stem abrasions. The objectives of this study were to evaluate the aggressiveness of diverse *R. solanacearum* isolates when applied to foliar tobacco plant parts during flower removal and to determine if an *Avr*-induced resistance response found with certain isolates in root tissue also occurs in stem tissue. The experiment was conducted at the Pee Dee Research & Education Center using the tobacco variety K346 and 23 isolates of *R. solanacearum* selected for differences in genetic diversity and aggressiveness. Plots consisted of a single row of 10 plants and the experimental design was a RCB with 4 replications. Inoculation simulated mechanical flower removal – a steel cutter blade was misted with a 1×10^6 cells/ml suspension of each isolate. A water inoculated treatment was used as the control. Plants were assessed weekly starting 21 days after inoculation. Stem necrosis was recorded at the final disease assessment date. Results showed significant differences in the amount of disease caused by the selected isolates of *R. solanacearum* when inoculated to foliar plant parts. The resistance mechanism functioning against tomato strains in root infections also appears to function in tobacco stem tissue.

Resistance to DMI fungicides in *Venturia inaequalis* from Pennsylvania
E. E. PFEUFER (1), J. W. Travis (1), H. K. Ngugi (1)
(1) Penn State University, Biglerville, PA, U.S.A.
Phytopathology 100:S100

Apple scab, caused by *Venturia inaequalis*, is the most economically important disease of apple in the eastern United States. Over the past 25 years, apple growers have relied on sterol demethylation inhibiting fungicides (DMIs) for scab control, but reduced efficacy has recently been noted. The aim of this study was to evaluate the sensitivity of *V. inaequalis* isolates from Pennsylvania to DMI fungicides. In 2009, leaves and immature fruit with scab symptoms were collected from 14 commercial orchards. Growers provided management history of the sampled plots. A total of 296 single-spore cultures were isolated from the tissues and maintained individually. Each isolate was tested for sensitivity to DMIs on 1/4-strength PDA plates amended with a range of concentrations of myclobutanil, fenbuconazole, or difenoconazole. Relative growth (RG) values were calculated and isolates with RG >75% on plates amended with 0.5 mg/ml were scored as resistant to the particular fungicide. About 14% of the isolates were cross-resistant to all three fungicides. Age of trees, size of orchard, number of DMI sprays in 2009, and lack of dormant copper sprays were positively correlated ($0.0001 < P < 0.05$) with the incidence of resistant isolates. Use of dormant copper sprays reduced the odds of an isolate being resistant to myclobutanil by about half (odds ratio = 0.446; 95% confidence interval = 0.239 to 0.832; $P = 0.011$). Management practices that reduce the risk of resistance to DMI fungicides in *V. inaequalis* were identified.

Control of Cylindrocladium black rot of peanut with Propulse, a 1:1 mixture of prothioconazole and fluopyram
P. M. PHIPPS (1), D. P. Tylenko (1), G. H. Musson (2)
(1) VPI&SU, Suffolk, VA, U.S.A.; (2) Bayer CropScience, Research Triangle Park, NC, U.S.A.
Phytopathology 100:S100

Two field trials were conducted in 2009 to assess control of Cylindrocladium black rot (CBR) of peanut with foliar sprays of Provost 433SC (prothioconazole 0.084 + tebuconazole 0.169 kg a.i./ha or prothioconazole 0.113 + tebuconazole 0.226 kg a.i./ha), and seed-furrow treatments with Proline 480SC (prothioconazole 0.2 kg a.i./ha) or Propulse 400SC (prothioconazole 0.215 + fluopyram 0.215 kg a.i./ha). All plots received three foliar sprays of Provost at the low or high rate and one final spray of chlorothalonil 1.263 kg a.i./ha. Treatments were mixed in water for delivery in 140 liter/ha in foliar sprays and 48 liter/ha in-furrow. The standard for CBR control was metam sodium 32 kg a.i./ha applied under each row 2-wk prior to

planting. Plots were four, 10.7-m rows spaced 0.9-m apart and treatments were randomized in four complete blocks. Low and high rates of Provost provided excellent control of foliar diseases but showed no differences in CBR incidence. Proline in-furrow significantly reduced CBR incidence by 43 and 33% while the Vapam standard reduced incidence by 52 and 55% in trial 1 and 2, respectively. Propulse in-furrow reduced CBR incidence by 83 and 57% in both trials. Yields were increased significantly by Propulse and by metam sodium in trial 1. Yields in trial 2 were not different; however, Propulse and metam sodium made the highest yields. Propulse is the first in-furrow fungicide to show promise as a replacement for metam sodium in control of CBR.

First report of *Fusarium oxysporum* f. sp. *lycopersici* race 3 on tomato in Iran

S. PIRAHESH (1), H. Zamanizadeh (1), S. Rezaee (1), B. Morid (2)
(1) Islamic Azad University-Science and Research Branch, Tehran, IRAN; (2) Islamic Azad University-Takestan Branch, Takestan, IRAN
Phytopathology 100:S101

Fusarium wilt of tomato, caused by Fusarium oxysporum f. sp. lycopersici, is a devastating disease in major tomato-growing regions worldwide and has been reported in at least 32 countries. Race 1 described in 1886 and race 2 was first reported in 1945. Race 3 was observed in Australia in 1978 and was subsequently reported in several U.S. states. Thirty five isolates of Fusarium oxysporum were morphologically identified from wilted tomato plants in greenhouses in Sistan and Baluchestan Province. The pathogenic types (forma speciales and races) of Fusarium oxysporum was identified with polymerase chain reaction (PCR) technique. the partial nucleotides sequence of polygalactoronase genes amplified with specific primer sets (uni, sp13 and sp 23). Results indicated that eleven isolates could be classified as Fusarium oxysporum f. sp. lycopersici, race 3. This technique could be helpful in epidemiological and etiological studies to monitor the behavior of the pathogen in disease plants. This is the first report of presence of Fusarium oxysporum f. sp. lycopersici race 3 on tomato in Iran.

***In vitro* evaluation of native *Trichoderma* isolates against fungal trunk disease pathogens in Ensenada, Mexico**

J. A. Plata-Caudillo (1), C. Valenzuela-Solano (2), R. HERNANDEZ-MARTINEZ (1)
(1) Microbiology Department, CICESE, Carretera Ensenada-Tijuana No. 3918, Zona Playitas, Ensenada, Baja California, MEXICO; (2) INIFAP, Campo Experimental Costa de Ensenada, Ensenada, Baja California, MEXICO
Phytopathology 100:S101

In Mexico, Ensenada, Baja California produces around 95% of the wine. Trunk diseases, caused mainly by fungi are among the main factors limiting vineyard longevity and productivity in the area. Due to the importance of these pathogens, finding control strategies is a priority. Here, we isolated *Trichoderma* spp. from field-grown grapevines, and evaluated their potential to control *L. theobromae* (Lt), *D. seriata* (Ds), *N. vitifusiforme* (Nv), *D. corticola* (Dc), *N. australe* (Na), *Cylindrocarpon* sp. (Cy), *P. aleophium* (Pa), and *P. chlamydospora* (Pc), under laboratory conditions. Six native isolates were compared with a commercial strain of *T. harzianum* (T-22). Percent of inhibition of the pathogens growth by T-22 vary from 43% to 50%, while inhibition by the isolates CCMT01-1 and SACH26-1, were significant higher for Na (75% and 96%), Ds (53% and 82%), Nv (66% and 79%), Dc (55% and 95%) and Lt (56% and 57%) respectively. CCMT01-1 and SACH26-1 were also capable of inhibit the growth of Pa and Pc by producing volatile and nonvolatile compounds. Based on their morphological characteristics and the analysis of their internal transcribed spacer region (ITS-5.8S-ITS2), CCMT01-1 and SACH26-1 were identified as members of *T. gamsii*. These results open the possibility to use CCMT01-1 and SACH26-1 as biological control agents of trunk diseases fungi affecting grapevines in the Ensenada Region.

Association of multiple virus infections with apple disease in western Colorado

R. POKHAREL (1), R. Mock (2), R. Li (2), G. Kinard (2), H. Larsen (1)
(1) Colorado State University, Grand Junction, CO, U.S.A.; (2) Beltsville, MD, U.S.A.
Phytopathology 100:S101

Gala and Golden Delicious apple trees with small leaves and small, deformed fruit were observed in an apple orchard in western Colorado. The symptoms on Gala were more severe than on Golden Delicious. To understand the possible association of viruses and viroids with the disease, three leaf and 20 fruit samples were collected from these trees, and asymptomatic Red Delicious trees, in October 2009 and tested for Apple chlorotic leafs pot virus (ACLSV), Apple mosaic virus (ApMV), Apple stem grooving virus (ASGV), Apple stem pitting virus (ASPV) and Cherry rasp leaf virus (CRLV) by RT-PCR, and Apple dimple fruit viroid, Apple fruit crinkle viroid, Apple scar skin viroid and Pear blister canker viroid by dot-blot hybridization. All five viruses were detected in fruit samples with different incidences, but no viruses were found in leaf samples. ACLSV was detected in all samples, and ASGV was detected in all Red Delicious and Golden Delicious trees but only in 3 of 8 Gala trees. CRLV was detected in all Golden Delicious samples, 7 of 8 Gala samples, and was not found in Red Delicious samples. ASPV was detected in 3 Golden Delicious and 1 Gala samples. ApMV was detected in only one Gala sample. Viroids were not detected in any samples. Asymptomatic Red Delicious trees were infected with only two viruses, while symptomatic Gala and Golden Delicious trees were infected with up to four viruses, suggesting a possible synergistic role of these viruses in symptom expression.

Taxonomic status of *Acanthorhynchus vaccinii*: reassessment of the identity of the cranberry pathogen *Physalospora vaccinii*

J. POLASHOCK (1), P. Oudemans (2), J. Crouch (3)
(1) USDA ARS, Chatsworth, NJ, U.S.A.; (2) Rutgers University, Chatsworth, NJ, U.S.A.; (3) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A.
Phytopathology 100:S101

Fruit rot of cranberry is caused by complex of phytopathogenic fungi. *Physalospora vaccinii* (Shear) Arx & E. Müll. is one of the most common and widespread causal species of fruit rot in both native and cultivated habitats. This species is easily identified from cranberry hosts based on characteristic perithecia, ascospores, and large appressoria. We have identified two distinct morphological types of *P. vaccinii* in North America that are easily differentiated by colony color (gray vs. white) and ascospore size and shape. Using molecular phylogenetics based on a six-gene dataset, it was clear that these distinct morphological types are evolutionarily distinct taxa that should be named as separate species. Surprisingly, the molecular data also showed that these fungi are unrelated to other members of the genus *Physalospora* (family Xylariomycetidae). Instead, both modern isolates and the type specimens of *P. vaccinii* fall within the family Sordariomycetidae. Based on these data, we propose to resurrect the genus name *Acanthorhynchus* originally applied to this group by Shear in 1922, to include the two species pathogenic to cranberry, *A. vaccinii* and *A. alba*.

Application of intragenic technology for development of disease-resistant potato

G. P. PONCIANO (1), C. M. Rommens (2), D. R. Rockhold (1), K. F. McCue (1), M. C. Whalen (3), W. R. Belknap (1)
(1) USDA ARS, Albany, CA, U.S.A.; (2) J. R. Simplot Company, Simplot Plant Sciences, Boise, ID, U.S.A.; (3) Pacific West Area USDA-ARS, Albany, CA, U.S.A.
Phytopathology 100:S101

Intragenics (also known as 'cisgenesis') is a plant transformation technology that consists of employing only genes, regulatory, and transfer DNA sequences from the plant genus to be transformed. Current status of our use of the technology to produce disease-resistant potatoes will be presented. The late blight disease caused by *Phytophthora infestans* continues to be potato's most serious disease worldwide. Using an intragenic transformation vector developed by J.R. Simplot Company, and the recently cloned RB resistance gene effective against a wide spectrum of *P. infestans* races, we aim to develop a late blight-resistant potato. We are also developing a strategy for intragenic potato plants resistant to viruses. We took a computational approach to identify virus homologous sequences present in the potato genome and in potato EST sequences. The identified genomic sequences are being evaluated to determine their potential to serve as si/miRNA templates to induce plant viral disease resistance via RNAi.

Application of a simple extraction method for the detection of viruses in plants and insect vectors

S. POOJARI (1), G. Karthikeyan (2), O. J. Alabi (3), T. A. Damayanti (4), K. Manoranjitham (5), N. Balakrishnan (6), R. A. Naidu (3)
(1) Washington State University, Prosser, WA, U.S.A.; (2) Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, INDIA; (3) Washington State University, Irrigated Agriculture Research and Exten Ctr, Prosser, WA, U.S.A.; (4) Faculty of Agriculture, Bogor Agricultural University, Bogor, INDONESIA; (5) Department of Fruit Crops, Tamil Nadu Agricultural University, Horticultural College and Research Institute, Coimbatore, INDIA; (6) Dept. of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, INDIA
Phytopathology 100:S101

Accurate diagnosis of plant viruses is critical for developing management strategies to mitigate their negative impacts. A wide range of methods have been used for the extraction of viral nucleic acid prior to detection by reverse transcription (RT)-PCR or PCR, depending on whether the test sample is infected with RNA- or DNA-containing virus, respectively. In this study, we used a relatively simple buffer for extraction of fresh leaf and fruit tissues and

for elution of nucleic acids from samples spotted on FTA® cards or nitrocellulose membranes. The extracts were used directly in one step-single tube RT-PCR or PCR for amplification of virus-specific DNA bands with species-specific primers. The amplicons were cloned and sequenced, and the derived sequences compared with corresponding sequences in the databases to confirm the presence of virus(es) in a given sample. The sample extraction buffer was also tested in combination with RT-PCR for the detection of tospoviruses in vector thrips and ampeloviruses in grape mealybugs. The practical utility of this protocol for the detection of RNA- and DNA-containing viruses will be discussed.

Timing and methodology of application of Azoxystrobin to control *Rhizoctonia solani* in sugarbeet

S. Pooran DeSouza (1), M. D. Bolton (2), M. F. KHAN (1)
(1) NDSU Plant Pathology, Fargo, ND, U.S.A.; (2) USDA - ARS, Fargo, ND, U.S.A.
Phytopathology 100:S102

Rhizoctonia solani AG 2-2 is the causal agent of Rhizoctonia root and crown rot of sugar beet (*Beta vulgaris*) in North Dakota and Minnesota. This disease is a major limiting factor to sugar beet production. Management strategies currently include using partially resistant cultivars and fungicides. Azoxystrobin is the most widely used fungicide for disease control. Our objective was to determine the best time for application of azoxystrobin and to compare the efficacy of azoxystrobin in foliar and soil drench applications in greenhouse trials. Azoxystrobin was applied as a hypocotyl drench at 0, 3, 10, 14, and 21 days post-inoculation, at 0, 7, 14, and 28 days pre-inoculation, and applied to either foliage or soil of inoculated sugar beet hypocotyls at the four-leaf stage. Based on disease severity means, azoxystrobin applied at pre-inoculation was not significantly different from non-inoculated check. Post inoculation applications at 0 and 3 days had low root disease severity and at 21 and 28 days disease severity were similar to inoculated checks. In efficacy trials, foliar application of azoxystrobin was significantly different from soil drench application and had the highest disease severity that was similar to inoculated checks. Azoxystrobin application as a soil drench at the base of the hypocotyls and prior to infection was effective in controlling disease.

Field efficacy of propiconazole on diverse *Sclerotinia homoeocarpa* population structures

J. T. POPKO (1), C. Ok (2), M. T. McGrath (3), G. Jung (2)
(1) Department of Plant, Soil and Insect Science, University of Massachusetts-Amherst, Hadley, MA, U.S.A.; (2) Department of Plant, Soil and Insect Science, University of Massachusetts-Amherst, Amherst, MA, U.S.A.; (3) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Riverhead, NY, U.S.A.
Phytopathology 100:S102

Dollar spot (*Sclerotinia homoeocarpa*) is a major turfgrass disease requiring fungicide application to maintain acceptable conditions for golf. Demethylation inhibitors (DMI) are the most frequently used fungicide class with confirmed resistance in *S. homoeocarpa*. Due to the quantitative nature of DMI resistance, a clear correlation between in vitro sensitivity values and reduced field efficacy has yet to be established for *S. homoeocarpa*. The study objective was to determine field efficacy of propiconazole rates on *S. homoeocarpa* populations with differing in vitro sensitivities. Prior to fungicide application, DMI sensitivity of populations on fairways of four golf courses and the UMass turf center was characterized by determining relative mycelium growth percentage (RMG%) on 0.1 µg a.i./ml propiconazole-amended medium. The UMass site consisted of a sensitive population (0–20% RMG) and the golf course sites consisted of two insensitive populations (50–80% RMG) and two with discrete bimodal populations (0–40% and 60–90% RMG). *S. homoeocarpa* was completely controlled with all propiconazole rates at the UMass site for all but one rating date. Reduced efficacy was observed at all four golf course sites on multiple rating dates, and 90% of isolates sampled 7 and 14 days after DMI application had RMG values >60%. Results indicate isolates with >60% RMG are likely responsible for reduced DMI field efficacy, despite quantitative variation of in vitro sensitivity values.

Effects of soybean cyst nematode on growth of kidney and navy bean

S. H. POROMARTO (1), B. D. Nelson (1)
(1) Dept. Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S102

Phaseolus vulgaris is a host of soybean cyst nematode (SCN; *Heterodera glycines*), but the effects of SCN on growth of plants are poorly studied. The effects of SCN (HG type 0) on cultivars Montcalm (kidney bean) and Mayflower (navy bean) were evaluated in the field in 2008 and 2009. Arveson loam soil was pasteurized and re-infested with 0, 1,000, 2,500 and 5,000 eggs/100 cc soil for Montcalm in 2008. In 2009 for Montcalm and Mayflower, treatments were similar but without the 1,000 eggs/100 cc. Soil was placed in 10 L plastic pots that were buried in the field with the bottoms removed. In 2008 there were no significant effects on growth of Montcalm in two field experiments, but in 2009, in a single experiment, pod weight (PW), seed number (SN), seed weight (SW), and total dry weight (TDW) of the above-ground-plants were significantly ($P < 0.05$) less at 2,500 and 5,000 eggs/100 cc soil compared with the control. There were also significant ($P < 0.05$) reductions of plant height (PH), PW, SN, SW, and TDW of Mayflower in 2009 in one field, but not a second field, when infested with 2,500 or 5,000 eggs/100 cc soil compared to the control. In both cultivars there were no significant differences among the two SCN treatments. SCN has now been shown to cause a yield loss in three dry bean classes and is a potential threat to the dry bean industry in the Dakota-Minnesota region.

Lack of adaption toward greater reproduction of soybean cyst nematode on dry bean

S. H. POROMARTO (1), B. D. Nelson (1)
(1) Dept. Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S102

Phaseolus vulgaris is a host of soybean cyst nematode (SCN; *Heterodera glycines*), a pathogen now in the major bean production area of North Dakota-northern Minnesota. The nematode reproduces less on most bean classes compared to soybean, but can still reduce plant growth. An important question is the following: will SCN adapt to dry beans and over time increase in ability to reproduce on roots? To answer this question the following experiments were conducted. The cultivars Premiere and Cirrus (navy), Buster and Othello (pinto), and Eclipse and Jaguar (black) were grown in 'Conetainers' in sand in plastic pots immersed in a water bath at 27 degrees C in the greenhouse. Seedlings were inoculated with 2000 eggs per plant of SCN HG 0 and cysts were harvested and counted after 40 days. The eggs were immediately extracted from those cysts and seedlings were inoculated again and grown for 40 days using the same methods. Soybean Lee 74 was used as a control. A female index (number of cysts produced on the test plant divided by the number of cysts produced on Lee 74) was calculated for each bean cultivar after each 40 days. This procedure was repeated until 8 generations of eggs were completed. There was no significant ($P \leq 0.05$) change overtime in the female index on the six bean cultivars. Therefore, no evidence of an adaptation toward higher reproduction on dry bean cultivars was detected during two 11 month periods of continual reproduction of HG 0 on roots.

Aggressiveness and management of metalaxyl-resistant *Pythium ultimum*

L. D. PORTER (1)
(1) USDA ARS, Prosser, WA, U.S.A.
Phytopathology 100:S102

Pythium ultimum is a major seed rotting pathogen of green pea in the Pacific Northwest, U.S.A. Metalaxyl (m), a systemic fungicide, is commonly used to manage seed rot due to *Pythium* spp. Recently, m-resistant isolates of *P. ultimum* were isolated from soil in ID, OR and WA. A m-resistant and m-sensitive isolate were recovered from each of five fields: a single field in ID, and two fields in WA and OR, respectively. Paired isolates were assessed for growth at 7.2 and 10°C on corn meal agar. These temperatures represent common soil temperatures when seeding green pea. Growth of m-resistant isolates was either signficantly greater or greater than the paired sensitive isolate at both temperatures in replicated tests, except for a single isolate in one of two trials at both temperatures. Faster growth rates for m-resistant isolates than for m-sensitive may indicate that resistant isolates are more aggressive than sensitive isolates in colonizing peas at these temperatures. Also, potential fungicide seed treatments (phosphorous acid, potassium silicate, metalaxyl, metalaxyl-M, fosetyl aluminum and cyazofamid) to manage seed rot by m-resistant isolates were assessed in infested soil at 10°C. Cyazofamid was the only fungicide that improved germination above 50% when soil was inoculated with a m-resistant isolate. Germination of seed treated with m or m-M was reduced by 58% when soil was infested with a m-resistant compared to a m-sensitive isolate of *P. ultimum*.

Biological control of seven woody invasive plant species with the fungal pathogen *Chondrosterium purpureum*

A. R. POST (1), C. A. Judge (2), D. Little (3), J. C. Neal (3), M. Benson (4)
(1) Virginia Tech, Blacksburg, VA, U.S.A.; (2) BASF Corporation, Dewitt, MI, U.S.A.; (3) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S102

The plant pathogen *Chondrostereum purpureum* is a wood rotting fungi currently registered in the United States and Canada as Chontrol™. It is intended for use in forests and on rights-of-ways as a biocontrol herbicide for invasive woody species. The objective of this study was to evaluate the effectiveness of cut-stump Chontrol applications against seven invasive woody species including empress tree, Chinese wisteria, Chinese privet, oriental bittersweet, red maple, tulip poplar and sweet gum. Trials were set up in a randomized complete block design with four to eight replications and four treatments plus a nontreated check. Treatments included cut-stump and wet-

blade applications of Chontrol in paste and liquid forms, as well as the industry standard against woody species, triclopyr. Each replication included plants of uniform stem diameter. All trials were repeated. Regrowth was measured 12 months after treatment for all species. No differences were observed between the stems treated with Chontrol and the nontreated check. Triclopyr controlled all species 100% with no regrowth observed. Though *Condrostereum purpureum* is documented to control some invasive woody species, current formulations and application methods failed to provide control of common woody invasive species in North Carolina. It is possible this is due to application timing or improper environmental conditions for infection.

Virulence factors in xanthomonads pathogenic on pepper increase *in planta* growth of *Xanthomonas perforans*

N. POTNIS (1), G. V. Minsavage (1), D. J. Norman (2), J. B. Jones (1)
(1) Department of Plant Pathology, University of Florida, Gainesville, FL, U.S.A.; (2) Mid-Florida Research & Education Center, University of Florida, Apopka, FL, U.S.A.
Phytopathology 100:S103

Comparative genomics has been widely used for identifying host specificity/virulence factors. Four *Xanthomonas* species pathogenic on tomato were sequenced. These include *X. euvesicatoria* (*Xcv*85-10) (already sequenced and published), and three draft sequences of *X. vesicatoria* (*Xv*1111), *X. perforans* (*Xp*91-118), *X. gardneri* (*Xg*101). *Xcv*8510, *Xv*1111 and *Xg*101 are also pathogenic on pepper, whereas, *Xp*91-118 is not. Comparison of genomes of pepper pathogens *Xcv*8510, *Xv*1111, *Xg*101 to *X. perforans* draft genome revealed a number of genes shared by pepper pathogens, which could be candidate virulence factors on pepper. We cloned three candidate genes and conjugated individually and in combination in ME24 (91-118*ΔavrXv3*), which no longer elicits an HR in pepper; however, *in planta* growth of ME24 is more similar to that of an avirulent strain than a virulent pepper strain. ME24 transconjugants carrying these genes showed increased *in planta* growth and also comparatively increased number of lesions on pepper cv. ECW when compared to ME24. The contribution of these genes towards pepper specificity is discussed.

Epidemiological studies on *Blackberry yellow vein associated virus*

B. POUDEL (1), W. M. Wintermantel (2), S. Sabanadzovic (3), I. E. Tzanetakis (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) USDA-ARS, Salinas, CA, U.S.A.; (3) Mississipi State University, Starkville, MS, U.S.A.
Phytopathology 100:S103

Blackberry production in the Southeast is threatened by blackberry yellow vein disease (BYVD), a disorder caused by virus complexes. Blackberry yellow vein associated virus (BYVaV), a recently identified crinivirus, is the most prevalent virus in the BYVD complexes. BYVaV is asymptomatic in single infection and acts synergistically during co-infecton with other viruses to cause disease. The objective of this study was to acquire information on the epidemiology of BYVaV including identification of initial sources of infection, vector(s) and alternate hosts. Several isolates of the virus infecting cultivated and wild blackberries were collected from different states with high BYVD incidence. The variability was determined after sequencing and analysis of four genomic regions of the virus; these regions being the most genetically diverse among viruses in the family *Closteroviridae*. Alternate host identification was performed by testing plant species present in blackberry fields with high BYVaV incidence. Whiteflies, known vectors of criniviruses, have been tested to identify species that can transmit BYVaV. The results of this study clarify factors contributing to the epidemiology of BYVaV, the primary component of the emerging BYVD, by identifying the geographical origin of the BYVaV, its potential vectors and alternate hosts.

The rpfF mutation of *Xanthomonas axonopodis* pv. *glycines* reduces bacterial pustule disease and activates defense in soybean

S. PRATHUANGWONG (1), W. Chuaboon (1), T. Chatnaparat (1), J. Thowthampitak (1)
(1) Kasetsart University, Chatuchack, Bangkok, THAILAND
Phytopathology 100:S103

RpfF is a protein similar to enoyl-CoA hydratase that synthesizes a diffusible signal factor (DSF) demonstrating as a virulence regulator in various plant pathogenic bacteria including *Xanthomonas axonopodis* pv. *glycines* (Xag), a cause of soybean bacterial pustule. Preliminary experiments reported that rpfF mutant of Xag failed to produce DSF, extracellular polysaccharide, siderophore, and many extracellular enzymes. In this study, we investigated the expression of rpfF on disease severity and defense response at the biochemical and transcriptional levels. Virulence assay was conducted by exploring the function of rpfF on soybean that symptoms and severity caused by the mutant were delayed and decreased respectively. Coinoculation of mutant and wildtype resulted in decreased pustule symptoms compared with wildtype alone inoculation. The relative accumulation of defense-related enzymes (POX, PAL, and β-1,3 glucanase), salicylic acid (SA) and pathogenesis-related proteins (PR-proteins) from plants treated with mutant and wildtype was greater upregulation compared to single inoculation. Result suggests categories of overrepresented genes expressing coordinately in response to rpfF mutant-wildtype interaction.

Evaluation of poultry litter for biocontrol of sclerotia of *Sclerotium rolfsii* in soil

R. PRATT (1)
(1) USDA ARS, Mississippi State, MS, U.S.A.
Phytopathology 100:S103

Poultry litter (PL), a major byproduct produced in large quantities on corporate poultry farms for which new uses are needed, was evaluated for potential use as a biocontrol material against sclerotia of *Sclerotium rolfsii* in soil. Survival of sclerotia was evaluated following their incubation within porous membrane filters in plates containing a sandy loam soil with and without PL amendments at 4 and 8% (dry weight equivalents). Sclerotia survived and germinated at relatively high percentages (70–100%) in Control soil after 2 wk, while survival and/or germination were consistently reduced in PL-amended soils. Physical destruction of sclerotia was usually greatest with 4% PL, whereas loss of viability of recovered sclerotia was usually greatest with 8% PL. Pre-incubation of soil with PL for 2 wk prior to addition of sclerotia gave less reduction in survival than occurred without pre-incubation. Incubation of sclerotia in soil for 4 wk rather than 2 wk reduced their survival in both Control and PL-amended soils. Results indicate that PL is efficacious for biocontrol of sclerotia of *S. rolfsii* in soil, and they suggest that different mechanisms of biocontrol may be involved at the 4% and 8% PL concentrations in soil.

Evaluation of a new method for collection and detection of plant pathogens within their vector

J. A. PRICE (1), A. Simmons (1), J. Bass (1), C. M. Rush (1)
(1) Texas AgriLife Research, Amarillo, TX, U.S.A.
Phytopathology 100:S103

Many of the important plant pathogens that affect agriculture are dependent on some type of vector for movement and distribution. Studies in epidemiology and vector/pathogen relations often involve detection of pathogens, not only within the host plant, but also within a single vector. *Wheat streak mosaic virus* (WSMV), which causes wheat streak mosaic, and *Candidatus* Liberibacter solanacearum (CLs), causal agent of Zebra chip, are two important plant pathogens that rely on vectors for their movement. However, analysis of a single vector can be challenging due to their small size. Therefore, a new method of pathogen testing is being investigated for detection of WSMV and CLs within a single wheat curl mite or psyllid, respectively. FTA membranes can be used to bind nucleic acids from both the vector and pathogen for long term storage, or go directly into analysis by real-time PCR without the use of traditional RNA/DNA extraction. This method has been used successfully in detection of WSMV and CLs within a single mite and psyllid, respectively. Tests are currently being conducted for detection of CLs within a single psyllid nymph collected from leaves displaying symptoms of Zebra chip. This method has potential as a powerful tool for analysis and quantification of plant pathogens within a single vector.

Relationships between *in vivo* and *in vitro* aflatoxin production: Reliable prediction of fungal ability to contaminate maize with aflatoxins

C. PROBST (1), P. J. Cotty (2)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) University of Arizona, USDA-ARS, Tucson, AZ, U.S.A.
Phytopathology 100:S103

Aflatoxins are highly carcinogenic mycotoxins frequently produced by *Aspergillus flavus*. Contamination of maize with aflatoxins imposes both economic and health burdens in many regions. Identification of the most important etiologic agents of contamination is complicated by mixed infections and varying aflatoxin-producing potential of fungal species and strains. In order to know the potential importance of an isolate to cause a contamination event, the ability of the isolate to produce aflatoxins on the living host must be determined. Aflatoxin production *in vitro* (synthetic and natural media) was contrasted with *in vivo* (living corn kernels) production in order to determine ability of in vitro techniques to predict the relative importance of causal agents of maize contamination. Several media and fermentation techniques were compared. There was no correlation between aflatoxin production on living corn and production in any liquid fermentation medium using any of the fermentation techniques. Isolates that produced aflatoxins on living corn frequently failed to produce detectable (limit of detection = 1 ppb) aflatoxin concentrations in synthetic media. Aflatoxin production on autoclaved corn kernels was highly correlated ($r^2 = 0.98$) with production on living corn kernels. The results have important implications for

researchers seeking to either identify causal agents of contamination events or to characterize atoxigenic isolates for biological control.

Subcellular localization of the replicase proteins encoded by a member of the family *Betaflexiviridae*

S. W. Prosser (1), C. Li (1), B. MENG (1)
(1) Dept. Molecular and Cellular Biology, University of Guelph, Guelph, ON, CANADA
Phytopathology 100:S104

Grapevine rupestris stem pitting-associated virus (GRSPaV) is a member of the genus *Foveavirus*, family *Betaflexiviridae*. The GRSPaV genome contains five ORFs, with ORF1 encoding a replicase polyprotein typical of members of the Alphavirus superfamily. It is known that replication of RNA viruses occurs in distinctive structures called "replication complexes" that can only be formed via association with a cell membrane. It was shown recently that translocation of the replication complexes of *Tobacco mosaic virus* was achieved by the actomyosin network. As a first step in unraveling the nature of GRSPaV replication complex and its interaction with cellular structures, we studied the subcellular localization of the full-length and truncated versions of its replicase polyprotein via fluorescence protein tagging and microscopic observation. We discovered that the replicase polyprotein forms distinctive bodies resembling the replication complexes in tobacco cells. We further revealed that a subdomain of 76 amino acid residues in the methyl-transferase (MTR) is responsible for formation of these bodies. Interestingly, these punctate bodies appear to align with the microfilaments. We are testing the possibility that these bodies may traffick on the microfilaments using the model plant *Nicotiana benthamiana* co-expressing autofluorescent protein fusions to MTR and actin marker. This is the first report on the subcellular localization of replicase proteins encoded by a member of the Betaflexiviridae.

Validation and haplotyping of Fusarium head blight resistance genes in a diverse spring wheat germplasm

K. D. PURI (1), S. Chao (2), H. Bockelman (3), M. Mergoum (4), S. Zhong (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND, U.S.A.; (3) USDA-ARS National Small Grains Collection, Aberdeen, ID, U.S.A.; (4) Department of Plant Sciences, North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S104

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schw., is the most destructive disease of wheat and barley in North America. Use of host resistance is one of the most efficient and economic strategies for managing the disease. However, the FHB resistance sources used in the breeding programs are very limited. In this study, we haplotyped 83 PI accessions obtained from the National Small Grains Collections with various levels of FHB resistance at 17 molecular marker loci associated with the known FHB resistance QTLs on the chromosomes 2B (*Triticum carthlicum* 'Blackbird'), 3A (*T. aestivium* 'Frontana' and *T. dicoccoides* 'Israel A'), 3B (*T. aestivium* 'Sumai 3' and 'Wanshuibai'), 5A ('Sumai 3' and 'Frontana'), 6B ('Blackbird' and 'Wangshuibai'), and 7A (*T. dicoccoides*). Fifty three of the PI lines showed different haplotypes at the marker loci on 3B and 5A of Sumai 3 origin, suggesting that they may carry different FHB resistance genes from Sumai 3, the most frequently used FHB resistance source in spring wheat breeding programs. Marker data further showed that 22 of these lines also lacked the markers associated with FHB resistance in other known sources investigated. All the wheat lines have been evaluated for FHB resistance under greenhouse and field conditions and the results will be presented. The novel FHB resistance genes carried by the spring wheat lines could be utilized to develop new wheat varieties with enhanced FHB resistance.

Molecular studies of genotype, organ and physiological stage dependent immunity against karnal bunt (*Tilletia indica*) of wheat (*Triticum aestivum*)

S. PURWAR (1), S. Sundaram (2)
(1) Allahabad University, Allahabad, INDIA; (2) Center of Biotechnology, Nehru Science Center, Allahabad, INDIA
Phytopathology 100:S104

Tilletia indica. an economically important disease -Karnal bunt (KB) of wheat which is incited by a semi-biotrophic pathogen. It has been elucidated that various enzymes of phenylpropanoid pathway not only enhance structural defense and induce systemic acquired resistance but also inhibit PCD involved in pathogenesis. Besides it, there is also involvement of cytokinins which are interestingly involved in both suppression of PCD and induction of systemic acquired resistance. The observation that resistant wheat varieties have high level of ABA, which is a precursor of JA has clearly demonstrated that the JA dependent pathways play important role in triggering induced resistance against the KB pathogen. The activity of protease was higher in initial stages of resistant genotype and gradually declined in later stages after anthesis. However, in contrast to protease activity, reverse trend has been observed for cystatin levels in all the stages of resistant wheat spikes showing negative correlation to each others. Therefore, stoichiometric balances of protease and protease inhibitor play key role in controlling the resistance against KB probably by intervention of PCD.

***Pseudomonas savastanoi* found in association with stem galls on mandevilla**

M. L. PUTNAM (1), M. Curtis (1), M. Serdani (1), A. J. Palmateer (2)
(1) Oregon State University, Corvallis, OR, U.S.A.; (2) University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S104

Mandevilla splendens is a woody twining vine in the family Apocynaceae. Rooted cuttings with stem galls were submitted to the Oregon State University Plant Clinic and the Extension Plant Diagnostic Clinic in Homestead, FL for diagnosis. The concern was crown gall, but symptoms were not typical of that disease. Galls were around 0.5 in diameter, occurred above soil level near the stem base, often at mid-stem. Vertical cracks with oozing bacteria were abundant. Isolations for *Agrobacterium tumefaciens* were negative, as were PCR assays using *Agrobacterium* specific primers A, C' and E'. Isolations produced an abundance of fluorescent bacteria which were identified by substrate utilization (Biolog, Haywood, CA) to the genus *Pseudomonas*. PCR using DNA extracted from gall tissue and the IAALF/IAALR primer pair, which target a region of the *iaaL* gene (synthesis of 3-indoleacetyl-ε-L-lysine) unique to *Pseudomonas savastanoi* pv. *savastanoi*, yielded a ~454 bp product. This PCR product was sequenced and showed 100% identity (395/395 nucleotides) to the *P. savastanoi* pv. *savastanoi* strain NCPPB-3335 *iaaL* gene. To our knowledge, this is a new host family for *P. savastanoi* in the United States and the first time *P. savastanoi* has been associated with galls on mandevilla. The conspicuous nature of symptoms and the economic importance of this plant in the ornamental industry suggest that this disease may become significant.

Factors contributing to abscisic acid-mediated predisposition to disease caused by *Phytophthora capsici*

M. F. PYE (1), T. V. Roubtsova (1), M. V. DiLeo (2), J. D. MacDonald (1), R. M. Bostock (1)
(1) University of California, Davis, CA, U.S.A.; (2) Ithica, U.S.A.
Phytopathology 100:S104

Plants respond to changes in the environment with complex signaling networks controlled in part by phytohormones that display positive and negative downstream crosstalk. Disease response pathways are influenced by systemic increases in abscisic acid (ABA), such as occurs following brief dehydration stresses. Experiments with ABA-modified tomato plants indicate that ABA plays a critical, and perhaps dominant, role in predisposition to *Phytophthora capsici*. To further assess ABA's contribution relative to other factors in root stress-induced predisposition, this study examines how other phytohormones influence disease severity following an episode of salt stress, and if plants expressing anti-apoptotic genes are altered in their predisposition phenotype. Ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) were studied using tomato perception and deficient mutants. Several anti-apoptotic transgenes with different modes of action were examined for their potential to affect predisposition. ET, which can exacerbate disease symptoms in plant-microbe interactions, did not contribute to predisposition in our assay. JA- and SA-deficient mutants displayed a more severe disease phenotype than their respective wildtype backgrounds in both control and salt stressed treatments. Increased levels of ABA following salt stress might perturb SA and JA signaling to enhance predisposition of mutants already compromised in these defense signaling networks.

Genome sequence and comparative genomics of *Pseudomonas savastanoi* pv. *glycinea*

M. QI (1), D. Wang (1), C. A. Bradley (1), Y. Zhao (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S104

Bacterial blight, caused by *Pseudomonas savastanoi* pv. *glycinea* (*Psg*), is a common bacterial disease of soybean and occurs in most soybean growing areas. We have previously identified an ancestral soybean line which confers resistant to both *Psg* race 4 and a recently isolated strain B076. In an effort to identify effectors responsible for inducing resistance in the ancestral line, the genomes of both *Psg* strains were sequenced using 454 pyrosequencing. The genomes of both *Psg* strains consist of a single circular chromosome of more than 5.8Mb. In addition, *Psg* race 4 has two large (110 and 55 kb) and three small (all less than 10 kb) plasmids. For strain B076, it contains six large plasmids with a size of 130, 85, 80, 67, 50, and 40 kb, respectively.

Comparative genomic analyses between two Psg strains and with other sequenced pseudomonads revealed that the genomes of *Psg* strains are more closely related to that of *P. savastanoi* pv. *phaseolicola* 1448A than to *P. syringae* pv. *tomato* DC3000 or pv. *syringae* B728a. Though conserved, genome rearrangements and recombination events occur commonly within the two *Psg* genomes. Type three secretion gene clusters of *Psg* strains are near identical with that of strain 1448A. Coronatine biosynthetic cluster is present in strain B076, but not in race 4. Furthermore, candidate effectors have been identified from the two *Psg* genomes and determined whether they are responsible for inducing hypersensitive response in the ancestral line.

Identification of molecular markers associated with resistance to TSWV through genetic mapping

H. QIN (1), Y. Li (2), Y. Guo (3), G. He (4), C. Chen (5), A. Culbreath (6), S. J. Knapp (3), D. R. Cook (7), C. C. Holbrook (8), M. Wang (9)
(1) USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA, U.S.A.; (2) Department of Plant Pathology, University of Georgia, Athens, GA, U.S.A.; (3) Center for Applied Genetic Technologies, the University of Georgia, Athens, GA, U.S.A.; (4) Center for Plant Biotechnology, Tuskegee University, Tuskegee, AL, U.S.A.; (5) USDA-ARS, National Peanut Research Laboratory, Dawson, GA, U.S.A.; (6) Department of Plant Pathology, University of Georgia, Tifton, GA, U.S.A.; (7) Department of Plant Pathology, University of California-Davis, Davis, CA, U.S.A.; (8) USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA, U.S.A.; (9) USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, U.S.A.
Phytopathology 100:S105

Peanut is vulnerable to a range of diseases, such as tomato spotted wilt virus (TSWV), and early and late leaf spots. The objective of this study is to construct a genetic linkage map to facilitate quantitative trait locus (QTL) analysis and gene tagging for use in a marker-assisted breeding. Tifrunner has been released as a resistant cultivar to TSWV and leaf spots. New breeding line NC94022 has been identified with the highest resistance to TSWV. Two genetic mapping populations have been developed, a total of 248 F2:7s for Tifrunner x GT-C20 (T) and 352 F2:7s for SunOleic 97R x NC94022 (S). A total of 4574 simple sequence repeat (SSR) markers have been collected and screened among the parents of the populations for polymorphisms. Of the total SSR primer pairs, 269 and 173 primer pairs (markers) were polymorphic in these populations, respectively, and used in genotyping these RIL populations. Genotypes of the S population has been completed and the linkage map has been constructed which has 20 linkage groups (LG) with 186 mapped loci (173 SSRs and 13 with two loci). In 2009, we conducted field evaluation of F2:5s for disease resistance to TSWV with two replications, and one QTL for TSWV resistance have been identified with 2009 phenotype data. The phenotypic variation explained by this QTL was 40%. The seeds have been increased, multiple field phenotypes will be conducted in 2010, and the QTLs will be reevaluated. This map will be compared with the T population.

Nematicidal activity of *Gymnoascus reessii* za-130 and the properties of its nematicidal substance

J. QIU (1), J. Liu (1), T. Liu (1)
(1) Institute of Plant and Environment Protection, BAAFS, Beijing, PRC PEOPLES REP OF CHINA
Phytopathology 100:S105

Strain za-130 of *Gymnoascus reessii* was isolated from soil in Beijing suburbs and it had ability to kill the root knot nematode (*Meloidogyne incognita*) obviously. The nematicidal activity of the broth filtrate from za-130 against *Meloidogyne incognita* was determined with the mortality of *M. incognita* J2, the inhibition rates of the hatching amount of egg masses and individual eggs by the method of immersion. The results showed the corrected mortality of J2 was over 80% and would be reached to 100% when treated 24 hours. The hatching of individual eggs and egg masses were suppressed by broth of za-130 which had been condensed 5 times, with inhibition efficacies over 85% and 95% respectively, and the treated result of the masses one more efficacy than individual one is the first report. No more differences for the ability of killing compared with pesticide of Avermectin and Fosthiazate. The nematicidal substance from za-130 displayed a higher stability to heat when the temperature under 60°C after 2 hours and still have higher ability to against nematode with the inhibiting rates of 80% when the temperature over 75°C up-to 100°C. The tolerant ranges of pH from 1 to 8 have no affect for the ability of the broth and will lost the ability on pH 10. The broth could against ultraviolet radiation when the irradiation under 4 hours and will lost all actives after 8 hours. Treated with sunlight showed have no affect for the active of broth.

A new rapid assay for detecting tebuconazole resistance in *Cercospora arachidicola*

J. Qiu (1), A. K. Culbreath (1), K. L. STEVENSON (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S105

The demethylation inhibiting (DMI) fungicide tebuconazole is widely used in Georgia to control early leaf spot of peanut, caused by *Cercospora arachidicola*. In recent years, reports from Georgia and neighboring states indicated that tebuconazole was less effective than it used to be, although control failures have not been widespread. The objective of this study was to develop a rapid assay to detect tebuconazole resistance in *C. arachidicola*. In this assay, conidia were transferred directly from lesions to tebuconazole-amended medium and sensitivity was based on diameters of 3-day-old colonies. Isolates were collected in 2008 and 2009 from peanut fields with or without a history of DMI use. EC_{50} values were determined using the new assay and compared to EC_{50} values based on the standard mycelial growth assay in microtiter plates. For the new assay, EC_{50} values ranged from 0.39 to 6.17 µg/ml for 21 isolates in 2008 and from 0.36 to 9.73 µg/ml for 78 isolates in 2009. For the standard assay, EC_{50} values ranged from 0.017 to 4.65 µg/ml for 29 isolates in 2008 and from 0.025 to 5.56 µg/ml for 58 isolates in 2009. EC_{50} values were consistently higher for the new assay compared to the microtiter plate assay. For combined data from both years, there was a significant positive correlation between EC_{50} values from the two assays. The main advantage of the new assay is that it can be completed in 3 days, compared to 2–3 months for the standard microtiter plate assay.

Mechanisms of DMI resistance in field isolates of *Cercospora arachidicola*

J. Qiu (1), A. K. Culbreath (1), K. L. STEVENSON (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S105

Mechanisms of resistance to the demethylation inhibiting (DMI) fungicides in the early leaf spot pathogen, *Cercospora arachidicola*, were investigated. Based on mechanisms of DMI resistance reported in other fungi, mutations of the *CYP51* gene, which encodes the target 14 alpha-demethylase necessary for sterol biosynthesis, over-expression of *CYP51*, and active efflux of fungicide mediated by ATP-binding cassette (ABC) transporters were evaluated in relatively DMI-resistant and DMI-sensitive isolates of *C. arachidicola* collected from peanut fields. Sequencing of the CYP51 gene revealed alterations at codons 453 or 461 in 4 of the 10 DMI-resistant isolates. This is the first report of mutations in the *CYP51* gene associated with DMI resistance in *C. arachidicola*. However, based on a RT-PCR assay, *CYP51* expression in DMI-resistant isolates of *C. arachidicola* was not different from that in DMI-sensitive isolates. Except for one resistant isolate that became more sensitive to tebuconazole when promazine was added, there was no apparent increase in tebuconazole sensitivity in the presence of ABC transporter inhibitors flavonone or promazine. DMI resistance was not associated with over-expression of *CYP51* or activity of ABC transporters in the isolates tested. However, mutations in the *CYP51* gene were associated with DMI resistance in some *C. arachidicola* isolates, which can be used to develop PCR-based assays for detection of DMI resistance in populations of this pathogen.

The role of *Turnip crinkle virus* capsid protein in viral systemic movement in *Arabidopsis*

F. QU (1), M. Cao (1), X. Ye (2), K. Willie (3), J. Lin (1), A. Simon (4), M. Redinbaugh (3), T. Morris (2)
(1) OARDC Plant Pathology, Ohio State University, Wooster, OH, U.S.A.; (2) School of Biological Sciences, University of Nebraska, Lincoln, NE, U.S.A.; (3) USDA ARS, Wooster, OH, U.S.A.; (4) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S105

The capsid protein (CP) of *Turnip crinkle virus* (TCV) is a multi-functional protein needed for virus assembly and suppression of RNA silencing-based antiviral defense. In this report, we have examined genetic requirements for the different functions of TCV CP, and evaluated the inter-dependence of these functions. A series of TCV mutants containing alterations in the CP coding region were generated. These alterations range from single amino acid substitutions, domain truncations, to knockouts of CP translation. The latter category also contained two constructs in which the CP coding region was replaced by either the cDNA of a silencing suppressor of a different virus, or that of green fluorescence protein. These mutants were used to infect *Arabidopsis* plants with diminished antiviral silencing capability. There was a strong correlation between the ability of mutants to reach systemic leaves and the silencing suppressor activity of mutant CP. Virus particles were not essential for entry of the viral genome into vascular bundles in the inoculated leaves in the absence of antiviral silencing, but were necessary for egress of viral genome from the vasculature of systemic leaves. Our experiments demonstrate that TCV CP allows the viral genome to access the systemic movement channel through silencing suppression, and then ensures its smooth egress with virus particle assembly. These results illustrate that efficient long-distance movement of TCV requires both functions of CP.

Sequence analysis of *Raspberry latent virus* suggests a new genus of dicot infecting reoviruses

D. QUITO (1), R. R. Martin (2)
(1) Oregon State University, Corvallis, OR, U.S.A.; (2) Horticultural Crops Research Unit USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S106

Currently there are three assigned genera of plant reoviruses: *Phytoreovirus*, *Fijivirus* and *Oryzavirus*. With only two exceptions, all plant reoviruses infect monocotyledonous plants. The recent characterization of *Raspberry latent virus* (RpLV) isolated from red raspberry plants in northern Washington has revealed new dicotyledonous hosts for plant reoviruses. Phylogenetic analyses demonstrate that RpLV is related to reoviruses belonging to the genera *Oryzavirus*, *Cypovirus*, *Dinovernavirus* and *Fijivirus*. Analysis of the polymerase sequence showed 36% aa identity to *Rice ragged stunt virus* (RRSV, an oryzavirus). As a general rule, reoviruses with an aa sequence identity greater than 30% in the RdRp sequence are considered members of the same genus (two exceptions have been reported). The analysis of the 5′ and 3′ terminal regions, however, indicate that RpLV and RRSV have different conserved sequence motifs, which would suggest they are species from distinct genera. Furthermore, the first 3 nucleotides (AGU) at the 5′ terminus of RpLV are conserved among members of the genera *Cypovirus*, *Dinovernavirus*, and *Fijivirus*, supporting the relatedness among these reoviruses. The lack of conservation between the terminal sequences of RpLV and RRSV, the very low aa identity in sequences from segments S8 and S9, and the distinct hosts for these viruses justify the creation of a new genus for the classification of RpLV.

Screening of exotic and commercial sorghum accessions against a new virulent race (P6) of *Peronosclerospora sorghi* causing downy mildew disease

G. L. Radwan (1), R. Perumal (1), T. Isakeit (1), L. K. Prom (2), C. R. LITTLE (3), C. W. Magill (1)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, U.S.A.; (3) Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S106

A recent outbreak of sorghum downy mildew in Texas has led to the discovery of both metalaxyl resistance and a new pathotype (P6) in the causal organism *Peronosclerospora sorghi*. To identify new sources of resistance to P6, a total of 312 (245 mini-core lines representing diverse germplasm from ICRISAT and 67 elite commercial accessions from Kansas) were tested in a greenhouse inoculation study. Forty-eight mini-core and 20 Kansas accessions had < 10% infection and were selected as resistant for further confirmation. Out of the 48 mini-core accessions, 20 (IS1212, 4060, 4613, 4631, 5094, 12804, 12883, 12965, 22294(PMK108), 24453, 24462, 24463, 26617, 29314(PL512), 29358(PHN2), 29392(PHM36), 29606, 30092(AMM938), 30443, and IS30562) were photoperiod insensitive and showed 0% infection. Eleven of the commercial accessions (DKS28-05, DKS37-07, DKS44-20, DSS B64, MG4748, MG4665, MG4765, Pioneer 85Y40, Dyna-Gro 742C, NK5418, and TRX83774) showed 0% infection. All 245 mini-core germplasm accessions are also being screened in separate tests under greenhouse inoculation for the identification of anthracnose, head smut and ergot resistance sources. DNA fingerprinting has been completed with 60 SSRs (representing all ten sorghum linkage groups) using a high throughput ABI Prism 3100 DNA sequencing system to quantify genetic diversity among resistant accessions.

Survey of small RNA during the suppression of soybean defense responses by *Pseudomonas syringae* pv. *glycinae avrB*

O. RADWAN (1), B. Calla (1), L. Vodkin (1), M. Hudson (1), S. J. Clough (2)
(1) University of Illinois, Dept. Crop Science, Urbana, IL, U.S.A.; (2) University of Illinois, Dept. Crop Science and U.S. Department of Agriculture, Urbana, IL, U.S.A.
Phytopathology 100:S106

Bacterial effector proteins, secreted through type III secretion systems, have been shown to trigger defense responses when recognized by resistant plants, and to suppress defense responses in susceptible host plants. Here we examined the hypothesis that *Pseudomonas syringae* pv. *glycinae* (Psg) carrying the avirulence gene *avrB* suppresses soybean defense responses from a soybean host that lacks the corresponding *R* gene (*RPG1-b*). Gene expression profiling using soybean oligo microarrays indicated that while defense genes, transcription factors, genes involved in the phenoproponoid pathway and signal transduction components were induced in the incompatible reaction, they were suppressed in susceptible plants inoculated with *Psg* (*avrB*) compared to a *Psg* (*avrB*-) control. To address the possible role of small RNA during the suppression of defense responses in susceptible soybean, we examined the differential expression of small RNA (miRNAs and siRNAs) between resistant and susceptible plants inoculated by *Psg* carrying *avrB*. Among 39887 unique reads with high quality, 35594 reads were 19 to 24 bp in length. These sequences were blasted against soybean and *Psg* genome sequence databases to determine plant and bacterial origins and to narrow the lists of interest. This information is being used to identify possible targets of the siRNA and miRNAs. These analyses will provide insight into the possible role of small RNA during soybean-*Psg* interactions.

Inhibition of *Magnaporthe oryzae* using cells and cell-free extracts of several strains of *Bacillus*

A. RAHMAN (1), W. Uddin (1)
(1) Penn State University, State College, PA, U.S.A.
Phytopathology 100:S106

The suppressive ability of several isolates of *Bacillus subtilis*, *B. licheniformis* and *B. amyloliquefaciens* to *Magnaporthe oryaze*, the cause of gray leaf spot of perennial ryegrass, was evaluated through assessment of direct antagonism. Cells, culture supernatants and methanolic supernatant extracts of each isolate were tested *in vitro* for mycelial growth and conidial germination inhibition. Solid phase columns were used to extract hydrophobic/lipophilic compounds from the cell-free culture supernatants using different concentrations of methanol. Cells, culture supernatants, and methanolic extracts of all isolates of *B. subtilis* and *B. amyloliquefaciens* significantly inhibited mycelial growth. However, the inhibition zone of mycelial growth using cell-free culture supernatants and methanolic extracts were 20–35% smaller than that produced by live bacterial cells. The conidial germination of *M. oryzae* was reduced 70–80% by culture supernatants of *B. subtilis* and *B. amyloliquefaciens* isolates. *B. licheniformis* cells, culture supernatants and extracts had no significant effect on fungal growth or spore germination. The possible roles of specific components of methanolic extracts in induced systemic resistance in the perennial ryegrass-gray leaf spot pathosystem will be presented.

Effect of strawberry nursery infestation with *Colletotrichum gloeosporioides* on fruiting field Anthracnose crown rot severity

M. RAHMAN (1), M. E. Carnes (1), F. J. Louws (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S106

Anthracnose crown rot caused significant losses to strawberry (*Fragaria x ananassa*) nursery and fruit growers in the southeast U.S. in recent years. There is very little epidemiological information available to develop management strategies for this disease. Initial inoculum levels and spread in the nursery, impact of nursery inoculum on fruiting field disease severity and management options were studied for consecutive two years. Isolates from non-cultivated wild hosts such as Muscadine grape (Vitis rotundifolia) and Virginia Creeper (Parthenocissus quinquefolia) were pathogenic on strawberry and identified as potential sources of initial inoculum to the nursery. Assessment after 60 days of inoculating 5%, 10% or 25% mother plants in the nursery indicated inoculum load significantly ($P \leq 0.001$) impacted latent infection severity on adjacent daughter plants. Spatial dispersal data showed better fit using the empirical power law model compared to an exponential model based on R^2 and residual values. Steepness of the dispersal gradient suggested rouging of plants around infection foci could be used for removal/separation of infested plants from the healthy ones. Bare root plants from the 10% and 25% nursery inoculation levels caused significant ($P \leq 0.005$) crown rot incidence in grow-out studies in the fruiting field and reduced yield compared to noninoculated control or 5% inoculation level. Dipping infested plants in selected fungicides before fall planting significantly decreased crown rot.

Yield loss incited by orange rust (*Puccinia kuehnii*) on a highly susceptible sugarcane cultivar in Florida

R. N. RAID (1), J. C. Comstock (2), N. Glynn (2)
(1) University of Florida, Belle Glade, FL, U.S.A.; (2) ARS-USDA Sugarcane Field Station, Canal Point, FL, U.S.A.
Phytopathology 100:S106

Sugarcane orange rust, incited by *Puccinia kuehnii*, was initially reported in the Western Hemisphere in 2007, when it was first observed in Florida. Since that time, it has affected several commercial cultivars, notably CP80-1743, CP72-2086, and CL85-1040. Experiments were conducted to quantify the amount of yield losses on the latter, the most susceptible of these cultivars. Varying levels of orange rust were established at the Everglades Research and Education Center in Belle Glade, FL by applying the fungicide pyraclostrobin at 7, 14, 21, and 28 day intervals. Experimental units measured five rows by 15 m and were arranged in a randomized complete block design with eight replications. Non-sprayed plots served as controls. Rust severities were visibly estimated on the top-visible-dewlap leaf minus four using established assessment keys. The 2008 orange rust epidemic began in May and persisted through the remainder of the growing season. Disease severities ranged from near zero in the 7-day treatments to over 35% in the controls. Millable stalk

populations were reduced by 12% and stalk biomass by 32% when controls were compared with the 7-day treatments. Together, these accounted for reductions of 43% in tons of cane per hectare. Sucrose concentrations were significantly reduced by nearly 18% on this cultivar. Cumulative losses in terms of sugar per unit area of cane measured 53%, demonstrating the destructive nature of this disease under favorable conditions.

Evaluation of fungicides for management of downy mildew on sweet basil
R. N. RAID (1), E. McAvoy (2), D. D. Sui (3)
(1) University of Florida, Belle Glade, FL, U.S.A.; (2) University of Florida, Labelle, FL, U.S.A.; (3) University of Florida, West Palm Beach, FL, U.S.A.
Phytopathology 100:S107

Basil downy mildew, incited by the fungal pathogen *Peronospora belbahrii*, is a newly important disease of this popular herb in the U.S. First reported in Florida in 2007, the disease has since been reported in 17 states and Canada. While host-plant resistance to downy mildew has been observed among various basil species, most sweet basil varieties (*Ocimum basilicum*) are very susceptible. Multiple experiments were conducted at the Everglades Research and Education Center during 2009 to evaluate fungicides for downy mildew control. In one replicated field trial, 19 fungicides were evaluated under extreme disease pressures. The experiment consisted of a randomized complete block design and chemicals were applied foliarly at weekly intervals. Disease was visually assessed on a scale of 0 to 10, with 0 representing no disease and 10 representing the level of disease in the untreated check. Of the compounds tested, Revus (0.7), Pristine (1.0), Ridomil Bravo (1.0), Reason (1.0), Forum (1.0), BAS 651 (1.0), Quadris (1.7), and Ranman (1.7) provided for the highest levels of control, in that order. These were followed by Presidio (2.7), Gavel (4.0) and K-Phite (4.3), which provided intermediate levels of control. Of the remaining treatments, although Actigard (6.7), Bravo Weather Stik (6.7), Kocide 3000 (5.3), Previcur (7.7), Regalia (7.0), Serenade (7.0), and Tanos (6.0) provided for reductions in disease severity, the level of control was not commercially acceptable.

Self- and cross-interaction studies among Pelargonium flower break and Pelargonium line pattern viruses coat proteins and their domains
G. RAIKHY (1), S. M. Leisner (1)
(1) The University of Toledo, Toledo, OH, U.S.A.
Phytopathology 100:S107

Pelargonium spp., native to South Africa, are widely cultivated in Europe and North America as ornamentals. Vegetative propagation of Pelargonium subjects them to viral infections, which have detrimental effects on quality and quantity. Pelargonium flower break (PFBV) and Pelargonium line pattern (PLPV) are two most widespread viruses infecting Pelargonium spp. They both belong to the family *Tombusviridae* and have single-stranded positive-sense genomic RNAs of ~3900 nucleotides. The multifunctional, 37 kDa, coat protein (CP) is encoded by 3′ proximal ORF. CP of both viruses contains 3 different domains namely, R- (interacting with RNA), S- (virion shell) and P- (projecting). In this study, self- and cross-interactions of complete CPs of these two viruses along with identification of domain(s) involved is reported. Self- and cross- interaction between two CPs was observed by yeast two-hybrid analyses and confirmed by MBP pull-down assays. For PFBV, all the three domains showed self-association as well as interaction among themselves, and with full length CP. For PLPV, the interactions among different domains are under investigation though preliminary results show similar pattern to that of PFBV. When cross-interaction studies of the CP domain were performed, the S domains were identified to interact.

Characterization of Huanglongbing associated '*Candidatus* Liberibacter asiaticus' from citrus relatives
C. RAMADUGU (1), K. L. Manjunath (2), S. E. Halbert (3), R. H. Brlansky (4), M. Roose (1), R. F. Lee (2)
(1) University of California, Riverside, Riverside, CA, U.S.A.; (2) USDA ARS National Clonal Germplasm Repository for Citrus and Dates, Riverside, CA, U.S.A.; (3) Florida Division of Plant Industry, Gainesville, FL, U.S.A.; (4) University of Florida Citrus Research and Education Center, Lake Alfred, FL, U.S.A.
Phytopathology 100:S107

Citrus greening or Huanglongbing (HLB) is a devastating disease reported predominantly from *Citrus* species. Effective mitigation of HLB requires information on all possible means of distribution of the disease including spread by alternate hosts. Citrus relatives collected from HLB-infected regions of South Florida were analyzed for the presence of '*Candidatus* Liberibacter asiaticus' (LAS). qPCR of the 16s RNA region indicated the presence of LAS from several plant samples. Molecular confirmation of the presence of LAS was carried out by PCR amplification, cloning and sequencing of several other genomic regions of the bacterium. The taxonomic identity of the host plant materials was confirmed by comparing the sequence of a nuclear gene, malate dehydrogenase, with the sequence of known accessions from the Citrus Variety Collection, Riverside, CA. This is the first report of detection of the bacterium associated with HLB from naturally infected *Atalantia ceylanica* and *Severinia buxifolia* from the United States. The sequence information shows that *A. ceylanica* and *S. buxifolia* harbor a bacterium identical to LAS associated with HLB. The study helps in generating information about citrus relatives that can serve as alternate hosts for LAS. The nature of Liberibacters from other PCR positive citrus relatives was analyzed, and the importance of other hosts in management of HLB will be discussed.

Over-expression of the calmodulin gene SCaM-4 in soybean enhances resistance to *Phytophthora sojae*
S. RAO (1), M. El-Habbak (1), J. S. Haudenshield (2), D. Zheng (2), G. L. Hartman (2), S. S. Korban (2), S. A. Ghabrial (1)
(1) University of Kentucky, Lexington, KY, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S107

Soybean is the major oilseed crop in the world with an annual value of 14 billion dollars in the United States. Plant pathogens inflict heavy losses on soybean yield. One of the most important pathogens is the oomycete, *Phytophthora sojae*, that causes Phytophthora root and stem rot. A set of resistance genes (*Rps*) was found to confer resistance to the pathogen. However, due to selection pressure, virulent races continue to evolve. Calmodulin, a Ca^2–binding protein, is implicated in plant defense responses in different plant species. The cellular level of SCaM-4 is known to increase rapidly in response to pathogen infection. Over-expression of *SCaM-4* in transgenic tobacco has been correlated with enhanced broad resistance to pathogens. We used the bean pod mottle virus-based vector for over-expression of *SCaM-4* in soybean, which was verified by RT-PCR. The *SCaM-4* over-expressing plants exhibited a distinct phenotype compared with control plants. Mock, vector control, and *SCaM4* over-expressing Harosoy plants were inoculated with *P. sojae* race 3 using stem wound inoculation. While mock and vector control plants showed expanding necrotic lesions at the infection sites and many plants died within 10 to 15 days post-inoculation, all *SCaM-4* over-expressing plants remained vigorous, and showed only superficial small lesions at infection sites. Experiments are underway to silence the *SCaM-4* gene in P. sojae- resistant soybean cultivars to determine whether such treatment would jeopardize the resistance response.

Bacterial toxins as natural nematicides: A high-throughput screen using *C. elegans*
A. A. RASCON (1), A. Garcia (1), S. Hanson (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 100:S107

Plant parasitic nematodes cause devastating damage to a wide variety of crops throughout the United States and worldwide resulting in billions of dollars in crop losses annually. Various effective chemical methods of nematode control have been or are currently being phased out due to environmental and human health concerns. The loss of these nematicides is likely to lead to a resurgence in agricultural production losses caused by nematodes. Bacterial toxins such as Bt genes have been widely and successfully used as natural insecticides in agriculture. Therefore, the hypothesis is that bacterial toxins may be useful controls for nematodes. The data from a simple high-throughput toxicity assay using *Caenorhabditis elegans* to screen for naturally occurring soil bacteria that are toxic to *C. elegans* is reported here. To date, over 40 strains of bacteria have been identified that inhibit *C. elegans* growth and reproduction, and increase the mortality rate. Data on the identity of the nematicical bacteria and properties of the toxic molecules are included as well as discussion on the potential use of these toxins as nematode control agents.

A qPCR assay for detection and quantification of *Verticillium dahliae* in spinach seed
G. RAUSCHER (1), B. Mou (1), R. J. Hayes (1), S. T. Koike (2), K. Maruthachalam (3), K. V. Subbarao (3), S. J. Klosterman (1)
(1) USDA ARS, Salinas, CA, U.S.A.; (2) University of California Cooperative Extension, Monterey and Santa Cruz counties, Davis, CA, U.S.A.; (3) UC Davis, Davis, CA, U.S.A.
Phytopathology 100:S107

The fungus *Verticillium dahliae* is the causal agent of Verticillium wilt of lettuce and other specialty crops in the Salinas Valley of California. Spinach, another major specialty crop in California, is not affected by Verticillium wilt in commercial production. However, spinach seed infected with *V. dahliae* and planted in the Salinas Valley increases inoculum density and introduces exotic strains that may contribute to Verticillium wilt epidemics. The goal of this work is to develop a real-time quantitative PCR (qPCR) assay for the detection and quantification of *V. dahliae* in spinach seed. The assay is based on the use of SYBR Green methodology with previously published primer sequences specific for the β-tubulin gene of *V. dahliae*. Parallel plating and qPCR assays revealed that the qPCR assay can be used for reliable detection

of *V. dahliae* in seed infected at the 10 percent level. Because the qPCR assay enables rapid and reliable detection of *V. dahliae*, the assay has implications as a useful tool to limit the spread of the pathogen.

Detection and quantification of the sugar beet cyst nematode, *Heterodera schachtii*, through qPCR
G. RAUSCHER (1), K. Richardson (1)
(1) USDA ARS, Salinas, CA, U.S.A.
Phytopathology 100:S108

Heterodera schachtii Schm. is a major parasite of sugar beet (*Beta vulgaris* L.) worldwide. As few as two eggs per cubic centimeter are enough to cause significant economic damage. Classical diagnostic methods determine the number of cysts in soil samples, but are time consuming. The goal of this study was to design a quantitative PCR (qPCR) assay to more rapidly detect and quantify *H. schachtii* and facilitate pre-planting field detection. Primers for a multiple copy sequence (ITS2) and a single copy gene (β-tubulin) were designed and qPCR standardized using SYBR Green. Specificity and sensitivity of the reactions were tested using commonly found soil microorganisms. ITS2 primers amplify *H. schachtii* from different origins including New York and California, detecting as little as 10pg of nematode DNA per gram of soil and do not show cross amplification with other commonly found soil inhabitants. The β-tubulin primers show high specificity as well, with a lower detection threshold of 14pg/g. Correlation of results between the genes is 0.97. The described qPCR assay will provide breeders with a more sensitive and less variable method to distinguish responses of breeding lines to *H. schachtii*. Further applications of these tests have the potential to improve pest management programs.

Incidence of bacterial spot on pumpkins in Illinois
A. RAVANLOU (1), M. Babadoost (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S108

This study was conducted to assess incidence and severity of bacterial spot, caused by the bacterium *Xanthomonas campestris* pv. *cucurbitae* (Xcc), and variation among Xcc isolates in Illinois. Bacterial spot has become one the most devastating diseases of pumpkins in Illinois and other Midwest states. Xcc infects leaves and fruit, producing angular spots on leaves and small, circular lesions on fruits. Infected fruit with Xcc is easily colonized by *Fusarium* spp. and soft rot bacteria, resulting in rapid collapse of fruit. In the past four years, yield losses in some pumpkin fields in Illinois exceeded 60%. Jack-o-lantern pumpkins are more susceptible to Xcc than processing pumpkins. In 2009, 17 randomly-selected jack-o-lantern pumpkin fields were selected and incidence and severity of bacterial spot on leaves and fruit were assessed throughout the season. The fields were visited four times, at 3-week intervals. In each field, leaves and fruit in 12 locations, in an M-shaped sampling pattern, were evaluated. In each location, the incidence and severity of the disease were assessed on 10 leaves and five fruits. Average incidence of bacterial spot on leaves was 2.4, 4.2, and 5.0% in July, August, and September, respectively. The incidence of fruit infection ranged from 2 to 98%, with an average of 45.5%. Ten bacterial isolates from leaves and fruit from each field were collected and the variation in pathogenicity and genetics of the isolates are being investigated.

***Melaleuca quinquenervia* plants differ in susceptibility towards fungus *Puccinia psidii* infection and disease development**
M. B. RAYAMAJHI (1), G. Wheeler (1), P. D. Pratt (1), T. D. Center (1)
(1) USDA-ARS, Ft. Lauderdale, FL, U.S.A.
Phytopathology 100:S108

Puccinia psidii (rust fungus) attacks immature healthy foliage of *Melaleuca quinquenervia* (melaleuca), an invasive plant in southern Florida, U.S.A. Melaleuca plants grown under same growing conditions manifest either susceptible or resistant reactions towards this fungus. We hypothesize that the variable reactions may be due to the differences in terpenoid contents in melaleuca. This hypothesis was tested using screenhouse and field-grown melaleuca plants. Melaleuca seedlings were tagged, grown to ca 45-cm height, inoculated with uredospores, placed in screenhouse under rust-fungus infected trees and evaluated for symptoms during a 4-wk period. Plants were also evaluated for major terpenoid content. Crude terpenoids were tested for effects on uredospore germination. The percentage of seedlings that developed pustules (susceptible), halos only (resistant) and no halos or pustules (immune) were 63.07, 33.90, and 0.03, respectively; these trends were similar among field-grown plants. Terpenoids from neither nerolidol nor viridifloral types of plants inhibited uredospore germination. Gas chromatography analysis of susceptible plants showed significant increases in total terpenoid concentrations as well as some of its constituents (myrcene, limonene and Beta-caryophyllene) compared with resistant plants. These results suggest that terpenoid constituents influence rust-fungus disease development in melaleuca, though this interpretation needs to be confirmed through additional research.

***Burkholderia andropogonis* from citrus appears to have a functional *hrp* system and *pthA* and *pthB* from *Xanthomonas citri* enhance its pathogenicity**
G. REBELLO (1), D. Gabriel (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S108

Xanthomonas citri (Xc), the causal agent of citrus canker, can horizontally transfer *pthB*, an *avrBs3/ pthA* family type III effector gene on a self mobilizing plasmid, to other bacteria *in planta* (El Yacoubi et al., 2007). *Burkholderia andropogonis* (Ba), which has a relatively wide host range is the causal agent of bacterial brown leaf spot of citrus (Duan et al., 2009); Ba can therefore occupy the same niche as Xc. Since Florida's citrus canker eradication program was suspended in early 2006, there may be an increased opportunity for horizontal gene transfer involving Xc and Ba in Florida. Ba transformants which carried either *pthA* or *pthB* exhibited increased pathogenicity on citrus and also grew at least one log better than wild type in all citrus varieties tested, suggesting that the PthA and PthB effectors act in part to suppress host defenses. Type III effectors require a Type III secretion system (T3SS) in order to be delivered into plant cells. Southern blots using *hrcC* suggested the presence of a functional T3SS in Ba, and *hrcV* was amplified, cloned and sequenced from Ba. A *hrcV* knockout mutant of Ba failed to cause a hypersensitive response in nonhost tomato and was much less pathogenic on citrus as compared to wild type. Complementation experiments are in progress.

Effect of maize kernels maturation on transcriptional activity in *Aspergillus flavus*
B. N. REESE (1), G. A. Payne (2), C. P. Woloshuk (1)
(1) Purdue University, Department of Botany and Plant Pathology, West Lafayette, IN, U.S.A.; (2) North Carolina State University, Plant Pathology, Raleigh, NC, U.S.A.
Phytopathology 100:S108

Aspergillus flavus infects maize kernels in the field and contaminates them with aflatoxin. To better understand how the fungus responds to the kernel environment during infection, we analyzed gene transcription after growth of the fungus on kernels at four developmental stages (blister, milk, dough, dent). Five days after inoculation, total RNA was isolated from kernels and hybridized to Affymetrix Gene Chip arrays containing probes representing 14,163 *A. flavus* genes. Statistical comparisons of the expression profile data revealed significant differences ($P < 0.01$) that included unique sets of up-regulated genes for each kernel stage and six patterns of expression over the four stages. Among the genes expressed in colonized dent kernels were a phytase gene and six putative genes involved in zinc acquisition. A knockout-mutant of the phytase gene, *PHY1*, was obtained. Although growth of the mutant on medium containing inorganic phosphate was the same as the wild type, growth of the mutant was severely restricted when phytate was the sole source of phosphate. Further, growth after 5 days on maize ears (based on ergosterol content) and aflatoxin production of the mutant were reduced 50% compared to the wild type. The results indicate that phytase in *A. flavus* may have an important role in pathogenesis.

Dissecting cyst nematode CLE perception in Arabidopsis roots
A. REPLOGLE (1), J. Wang (1), J. Smeda (1), M. G. Mitchum (1)
(1) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S108

Cyst nematodes cause billions of dollars in crop damage by inducing enlarged, multinucleate feeding cells in host roots that serve as the sole nutrient source for the nematode to complete its life cycle. The feeding site, which is known as a syncytium, is formed in response to nematode secreted effector proteins. *Heterodera* species produce CLAVATA3(CLV3)/ESR (CLE) effector proteins sharing functional similarity with plant CLE signaling peptides involved with several aspects of plant development including maintenance of stem cell pools in the root meristem. Previously, we identified nematode CLE receptor candidates by screening mutants of plant CLE receptors and other closely related LRR-RLKs for resistance to synthetic *Heterodera* CLE peptide treatment. Mutants of *CLV2*, *CORYNE* (*CRN*) and *BARELY ANY MERISTEM* (*BAM1*) were resistant to peptide treatment and used in nematode infection assays and overexpression studies to reveal that CLV2 and CRN appear to be major players in nematode CLE perception. Further characterization of the defects in syncytium formation on the receptor mutants will help elucidate the role CLE peptides play in nematode parasitism.

Testing for mycotoxins using LC-MS/MS
J. RICHARD (1), D. Houchins (1), C. Brewe (1), M. Prinster (1)
(1) Romer Labs, Inc., Union, MO, U.S.A.
Phytopathology 100:S108

Mycotoxins have traditionally been detected by a variety of methods, including rapid methods (test kits) and reference methods, such as HPLC and GC. Of the reference methods, GC can be limited due to the necessity of derivatizing the compounds of interest. This may also be required for some HPLC methods. However, liquid chromatography may be used in conjunction with a variety of detectors, including fluorescence, UV-VIS, and others, including mass spectrometers. The coupling of liquid chromatography with a mass spectrometer (or tandem mass spectrometers, LC/MS/MS) allows for methods which are applicable to a wide variety of analytes, with no limitations by molecular mass, a straightforward sample preparation, and no chemical derivatization required. These methods also have the benefit of providing structural information on the target compound and the possibility of testing for many analytes in one run. Matrix effects and the effects of variations in sample preparation may be eliminated by the use of stable isotope-labeled internal standards. These features make LC/MS/MS methods useful and versatile in the detection of mycotoxins.

Functional characterization of a transcription factor family in *Fusarium verticillioides*

J. B. RIDENOUR (1), C. P. Woloshuk (2), W. Shim (3), B. H. Bluhm (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Purdue University, West Lafayette, IN, U.S.A.; (3) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S109

Fusarium verticillioides is a ubiquitous pathogen of maize, attacking seedlings, kernels, and stalks. Additionally, *F. verticillioides* produces fumonisin mycotoxins. Currently, little is known at the molecular level regarding pathogenesis or mycotoxigenesis in this important fungus. The goal of this study was to identify genes and regulatory mechanisms underlying pathogenicity and fumonisin biosynthesis in *F. verticillioides*. To this end, an entire family of transcription factors - CCAAT-binding factors (CBFs) - was functionally characterized through reverse genetics. The genome of *F. verticillioides* is predicted to contain six CBF-encoding genes, all of which were disrupted utilizing split-marker homologous recombination. For each mutant, morphology, pathogenesis, kernel colonization, and fumonisin biosynthesis were analyzed. The mutants displayed a range of morphological abnormalities, including reduced growth and conidiation. Furthermore, at least one of the genes was required for wild-type levels of kernel colonization, and at least two of the genes were required for wild-type levels of fumonisin biosynthesis. This study is one of the first systematic analyses of fungal CBFs in the context of plant pathogenesis and one of the first successful attempts to characterize an entire family of transcription factors in *F. verticillioides*. Additionally, these findings provide new insight into the regulation of pathogenesis and fumonisin biosynthesis in *F. verticillioides*.

Tomato grafting to manage bacterial wilt (caused by *Ralstonia solanacearum*) in the southeastern U.S.

C. L. RIVARD (1), R. M. Welker (1), S. O'Connell (1), M. M. Peet (2), F. J. Louws (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) United States Department of Agriculture, Washinton, DC, U.S.A.
Phytopathology 100:S109

Ralstonia solanacearum causes severe losses to tomato growers who manage infested soils in the southeastern U.S. Commercial tomato varieties offer little host resistance and fumigation has limited efficacy as primary inoculum can be re-introduced through soil water movement. Bacterial wilt (BW) is managed worldwide by grafting popular fruiting varieties onto resistant rootstocks. However, few rootstocks are available in the U.S. and little is known regarding their efficacy against native Ralstonia strains. Field trials were conducted in NC from 2007–2009 to determine the utility of grafting to manage BW and evaluate commercially-available rootstocks against endemic populations of R. solanacearum. Four rootstocks displayed effective partial resistance, and BW AUDPC values were significantly lower among all rootstocks compared to non- and self-grafted controls in all trials ($P < 0.05$). Fruit yield was significantly affected by grafting onto resistant rootstock ($P < 0.05$). Interestingly, several rootstocks showed differential efficacy across locations, suggesting that pathogen population dynamics plays a role in rootstock selection. 'RST-04-105' had complete resistance in eastern NC, but showed intermediate resistance in western NC. Similarly, 'Dai Honmei' had high resistance in western NC, but was intermediate in eastern NC. Grafting is an effective management strategy against bacterial wilt and could be an important component of a successful IPM program.

Anthracnose disease of annual bluegrass as affected by light-weight rolling and equipment traffic

J. A. ROBERTS (1), J. A. Murphy (1), B. B. Clarke (1)
(1) Rutgers University, New Brunswick, NJ, U.S.A.
Phytopathology 100:S109

Light-weight rolling is a common management practice employed to smooth the playing surface and increase ball roll distance (BRD) on golf course putting greens. Anthracnose is a serious disease of annual bluegrass [ABG; Poa annua L. f. reptans (Hausskn) T. Koyama] putting green turf caused by Colletotrichum cereale Manns. Light-weight vibratory rolling has been shown to reduce anthracnose severity on ABG, but the impact of other roller types or location of equipment traffic on this disease is unknown. A 3-yr field trial was established in New Brunswick, NJ in 2006 to evaluate the influence of roller type (i.e., sidewinder, vibratory and non-rolled) and location (center or perimeter) of equipment traffic on anthracnose severity and BRD of ABG turf maintained at 3.2 mm. The study was established as a strip-plot design with 8 replications. Both roller types reduced disease severity 2 to 13% compared to non-rolled turf under moderate disease pressure in 2007 and 2008. The heavier sidewinder roller had less disease than the vibratory roller on 4 of 13 rating dates and perimeter plots, which received increased equipment traffic, had less disease compared to center plots on 6 of 13 rating dates during this period. Results indicate that rolling can be used to increase BRD and improve turf quality without intensifying, and in some cases reducing, anthracnose severity on ABG putting green turf under moderate disease pressure.

Impact of hail damage during early reproductive stages on ear rot and mycotoxin contamination of maize

A. E. ROBERTSON (1), G. P. Munkvold (1), C. R. Hurburgh (1), S. M. Ensley (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S109

During the 2009 growing season in Iowa, two severe hail storms affected over 400,000 hectares of maize during early reproductive stages. Concerns were raised about increased ear rot disease and associated mycotoxin contamination. To address these concerns, samples of ears were collected within 48 hours of harvest from 57 fields damaged by hail and 25 undamaged fields. Ears were visually assessed for kernel damage and ear rot severity. After shelling, grain was ground and tested for deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM) using commercially available antibody-based lateral flow strip tests. Confirmation analysis on elevated samples was performed by Gas Chromatography for DON and High Pressure Liquid Chromatography for ZEA and FUM. Fusarium, Gibberella and Cladosporium ear rots were the most prevalent diseases. Hail damage to kernels increased the risk of ear rot. The most prevalent mycotoxin detected was DON (mean 2.63 ppm), followed by ZEA (mean 0.53 ppm) and FUM (mean 0.49 ppm). Levels of DON and ZEA in grain from hail damaged fields were greater than those detected in grain from undamaged fields. There was a positive relationship between ear rot severity and DON and ZEA contamination ($r = 0.645$, $P < 0.001$ and $r = 0.474$, $P < 0.001$, respectively). Levels of DON also were correlated with test weight ($r = –0.38$, $P = 0.004$) and protein ($r = 0.49$, $P < 0.001$). In the future, preharvest scouting of fields with suspected mycotoxins may be an effective strategy for targeting postharvest inspection and marketing activities.

Serological detection and molecular analysis of *Tobacco ringspot virus* and *Strawberry latent ringspot virus* in mint (*Mentha* sp.)

N. L. ROBERTSON (1), B. J. Furman (1)
(1) USDA ARS, Arctic and Subarctic Plant Genetic Resources Unit, Palmer, AK, U.S.A.
Phytopathology 100:S109

Mint (*Mentha* sp.) is commercially cultivated around the world by the food, medicinal, and landscape industries. Over 400 clonal mint accessions have been maintained by USDA, Agricultural Research Station in Corvallis, Oregon by the National Clonal Germplasm Respository since 1983, and more recently (2010) in Palmer, Alaska by the Arctic and Subarctic Plant Genetic Resources Unit. Due to an increased concern for viruses occurring in mint, 424 transplanted mint accessions in Palmer were assayed for selected viruses previously known to naturally infect mint. Leaves were collected from plants within four weeks of emergence and processed for ELISA and total RNA extractions. Assays were performed according to manufacturer's directions for the following ELISA kits: 1) Agdia, Inc. (IN) - *Alfalfa mosaic virus*, *Strawberry latent ringspot virus* (SLRSV), *Tobacco ringspot virus* (TRSV), and universal potyvirus, and 2) AC Diagnostics, Inc. (AR) - *Cucumber mosaic virus* and *Cherry rasp leaf*. ELISA results confirmed 13 SLRSV and nine TRSV singly infected plants and one plant with a double infection. Infected plants were confirmed by RT-PCR for all except one of the TRSV infected plants, and for only three SLRSV infected plants. Natural occurrence of TRSV in mint is limited to the United States while the distribution of SLRSV is worldwide. Maintenance of healthy mint plants is an important aspect when selecting plant material to send to requesting researchers.

Association of a phytoplasma with dieback in palms in Puerto Rico confirmed by nested-PCR assays

J. V. RODRIGUES (1), A. M. Vitoreli (2), A. L. Ramirez (3)
(1) University of Puerto Rico, San Juan, U.S.A.; (2) University of Florida, Gainesville, FL, U.S.A.; (3) Dept. Agriculture of Puerto Rico (CAPS), San Juan, U.S.A.
Phytopathology 100:S110

Diseases associated with phytoplasma infections are among the most serious phytosanitary problems in palms. Those diseases have the potential to adversely affect the landscape and tourism industry, where palms are key species, as well as the ecosystem. In Guaynabo, Puerto Rico, dieback and mortality of palms was observed, resembling symptoms described for lethal yellowing associated with phytoplasma. Samples from different species of palms were collected. DNA was extracted using DNeasy kit, and PCR was carried out using universal primers for amplification of phytoplasma DNA P1 and P7, and P1m/LY1623Sr. PCRs were followed by nested-PCR with two pairs of primers R16F2n/R16R2 and LY16-23Sf2/LY16-23Sr2. The amplified products were visualized in agarose gel/UV. A single fragment of 1.2Kb was observed in both primer combinations. Number and size of the fragment were expected for determining the occurrence of phytoplasma. All tests were repeated four times and confirmed by PDC-Regional Lab for the SPDN, Gainesville, FL. DNA from nonsymptomatic plants did not result in the amplification of PCR products. So far, detection of phytoplasma associated with symptoms similar to lethal yellowing of palms was confirmed for three species: Royal Palm (*Roystonea* sp.), Fishtail Palm (*Caryota mitis*), and Carpentaria (*Carpentaria acuminata*). To our understanding this is the first report of a phytoplasma occurring in palms in Puerto Rico.

Genetic diversity of barley spot blotch resistance sources by chromosome haplotyping

S. RODRIGUEZ (1), C. Torres Tuyo (1), P. Silva (1), A. Castro (1), C. Pritsch (1)
(1) University Uruguay, Montevideo, URUGUAY
Phytopathology 100:S110

Spot blotch, caused by *Cochliobolus sativus*, is a major foliar disease of barley that may become even more severe due to global climate change. Additional, independent, non redundant sources of resistance should therefore be incorporated into breeding programs to improve effectiveness and durability of new barley cultivars. Chromosome haplotyping at major QTLs for spot blotch resistance applied to candidate barley germplasm could contribute to identify valuable, non redundant resistant genotypes. Here, we analyzed a set of 25 diverse spot blotch resistant genotypes and 15 elite cultivars at QTLs *Rcs-qtl-1H-5-7* (four SSR markers, cv Morex as reference genotype), *Rcs-qtl-2H-7-8* (four SSR markers, cv Calicuchima as reference genotype), *Rcs-qtl-3H-1-3* (three SSR markers, Bowman BC as reference genotype) and *Rcs-qtl-7H-2-4* (two SSR and two STS markers, cv Morex as reference genotype). The number of haplotypes identified at each QTL varied between four (*Rcs-qtl-3H-1-3*) and ten (*Rcs-qtl-7H-2-4*). Moreover, nine resistance sources have no allele in common to cv Morex at *Rcs-qtl-7H-2-4*, while seven genotypes shared only one allele with Morex at QTLs *Rcs-qtl-1H-5-7*. Also, three genotypes shared no more than one allele with cv Calicuchima at *Rcs-qtl-2H-7-8* and three other genotypes shared no more than one allele to cv Bowman BC at *Rcs-qtl-3H-1-3*. These results contribute to our knowledge of useful, non redundant genetic diversity available within spot blotch resistant germplasm and may benefit barley breeding programs.

Arabidopsis thaliana* ecotypes with differential susceptibility to the bacterial pathogen *Xylella fastidiosa

E. E. ROGERS (1)
(1) USDA-ARS, Parlier, CA, U.S.A.
Phytopathology 100:S110

Pierce's disease of grapes and almond leaf scorch are devastating diseases caused by the bacterium *Xylella fastidiosa* (Xf). To date, progress in determining the 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 Xf. The long generation time and limited genetic resources for *Xylella fastidiosa* compound the problem. The model plant *Arabidopsis thaliana* is an ideal system for rapid progress in genetic and pathological studies. There are many publically available genetic resources for Arabidopsis and it has a short generation time. Arabidopsis has been evaluated as a model host for Xf and a pin-prick inoculation method has been developed. Following infection, Xf can be detected by microscopy and PCR. Xf has also been re-isolated from infected Arabidopsis tissue. Timcourses following Xf growth have revealed Arabidopsis ecotypes with differing susceptiblity to infection. The genetic inheritance of these differences is being investigated. Additionally, differences in gene expression between ecotypes following Xf infection will be presented.

Assessing relationships among isolates of *Wheat streak mosaic virus* using single nucleotide polymorphisms

S. ROGERS (1), U. Melcher (1), R. Allen (2), J. Fletcher (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) Oklahoma State University, Tulsa, OK, U.S.A.
Phytopathology 100:S110

Relationships among multiple isolates of a given plant pathogen species have been investigated by several molecular typing methods. The ability to extend the use of such typing strategies to assess previous spatial and temporal pathogen movement within a geographical area would facilitate disease management and forensic studies. We investigated the use of single nucleotide polymorphism (SNP) typing for assessing relationships among isolates of a plant virus within a specific geographical location. *Wheat streak mosaic virus* (WMSV)-infected wheat from nine locations within the western region of Montana was obtained from researchers at Montana State University. cDNA, generated from total RNA from infected wheat, was used as a template in the SNaPshot Multiplex System (Applied Biosystems). Fluorescent ddNMPs were added to the 3′-end of SNP-specific primers and the resulting sequences were separated by capillary electrophoresis. Each of the nine WSMV isolates produced a unique fingerprint for the SNPs, and a weak correlation between the fingerprints and the locations from which the isolates were collected was noted. The use of greater numbers of samples and assessment of isolates from additional geographical locations will allow more conclusive interpretations of SNP typing data and whether they could be useful for characterizing patterns of pathogen spread. This work represents the first application of SNP typing to plant pathogens for forensic purposes.

Assessment of *Strawberry mild yellow edge virus* infection in different ecotypes of the Chilean native strawberry *Fragaria chiloensis* (L.) Duch.

P. F. ROJAS (1), C. Sandoval (1), P. D. Caligari (1), R. R. Martin (2)
(1) Universidad de Talca, Talca, CHILE; (2) USDA-ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S110

Fragaria chiloensis ssp *chiloensis* (L.) Duch, is distributed naturally in Chile. Two botanical forms have been described; the white-fruited *chiloensis*, which is cultivated in coastal mountains between latitudes 35° and 39°S, and the small-red fruited *patagonica*, which grows widely between latitudes 35°and 47°S. *F. chiloensis*, the mother of the current commercial strawberry, produces berries with unique quality characters and has the potential to be used as a commercial berry. However, their yields can be severely affected by viral diseases. Aphid-borne viruses have been found in wild and cultivated *F. chiloensis*, but these plants do not show symptoms and the defense response mechanism in this species is unknown. The objective of this work was to study the development of SMYEV infection in different ecotypes of *F. chiloensis*. A SMYEV isolate from Chile was used to graft-inoculate three-month old healthy plants and a real-time PCR detection method was used for detecting the SMYEV RNA using CP specific primers. Our results showed virus detection at three days post inoculation (p.i.) in most *chiloensis* form ecotypes, whereas in the *patagonica* form the virus was not detected until six to eight weeks p.i. This suggests a differential response to SMYEV infection in the different genetic backgrounds. PR was supported by CONICYT and UTAL fellowships.

Survey and characterization of viral diseases affecting tomato crops in the north of Chile

I. ROSALES VILLAVICENCIO (1), P. Sepúlveda (1), C. Medina (1), M. P. Muñoz (1), C. Rojas-Bertini (2), R. Mora (1), M. Madariaga (1), J. K. Brown (3)
(1) Instituto de Investigaciones Agropecuarias, Santiago, CHILE; (2) Instituto de Investigaciones Agropecuarias, Arica, CHILE; (3) Department of Plant Science, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S110

During the past years the phytosanitary status of tomato cultivation in the north of Chile has declined due to the proliferation of several viral diseases. Various reasons have favored the introduction and proliferation of previously unrecorded viruses: the climatic conditions, the increase of the international commercial exchanges, and the introduction of *Bemisia tabaci* biotype B to the area. A survey was conducted in the Azapa Valley (Region of Arica and Parinacota) that included the main tomato viruses: Cucumber mosaic virus (CMV), Alfalfa mosaic virus (AMV), Pepino mosaic virus (PepMV), Tomato moisac virus (ToMV), Tomato spotted wilt virus (TSWV), Tobacco etch virus (TEV), Potato virus Y (PVY), Peru tomato mosaic virus (PTV) and the presence of begomovirus. Virus identification was based on enzyme-linked immunosorbent assay (ELISA) and PCR-based methods. After analyzing more than 1000 plants with virus-like symptoms, we have found no presence of CMV, AMV, ToMV, PVY, TSWV, and TEV. The more prevalent viral agents detected were PTV (9%), PepMV (27%) and the recently described begomovirus Tomato yellow vein streak virus (ToYVSV), detected in a 43%

of samples. ToYVSV is considered one of the most important begomovirus species currently affecting tomato and potato production in Brazil and Argentina (South-America), and it is the only begomovirus detected in the Chilean territory so far.

Xanthomonas albilineans needs an OmpA family outer protein for disease symptom development and multiplication in the sugarcane stalk

P. C. Rott (1), L. A. FLEITES (2), G. Marlow (2), M. Royer (1), D. Gabriel (2)
(1) CIRAD, Mixed Research Unit for Biology and Genetics of Plant Pathogen Interactions, Montpellier, FRANCE; (2) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S111

Xanthomonas albilineans (Xa) is a systemic, xylem-invading pathogen that causes sugarcane leaf scald. Xa produces albicidin, a potent antibiotic and phytotoxin which blocks chloroplast differentiation, thus causing the foliar symptoms of the disease. Albicidin is the only known pathogenicity factor in Xa, yet albicidin deficient mutants are still able to colonize the sugarcane plant. In an attempt to identify other major pathogenicity factors, we screened 1,216 independent Tn5 insertions in Xa strain XaFL07-1 by single inoculation onto sugarcane cultivar CP80-1743. Mutants were screened for reduced pathogenicity (i.e., capacity to induce leaf symptoms and to multiply in the sugarcane stalk). Five independent Tn5 insertions were found in gene XALc_0557, which is predicted to encode an OmpA family outer membrane protein. Each of these insertions resulted in a mutant strain that elicited very slight to no symptoms and was not able to move as efficiently within the sugarcane stalk, both spatially and in intensity, as wild type XaFL07-1. Additional phenotypic studies showed that these mutants: 1) produced albicidin, 2) were less motile and 3) were slower growing than the wild type Xa in vitro. However, these OmpA mutants were able to multiply in sugarcane leaf tissue to levels similar to the wild-type strain XaFL07-1. Complementation analyses are currently underway.

Complete 3′ end genome analysis of the asymptomatic *Citrus tristeza virus* isolate B192 and its eight symptomatic single aphid transmitted subisolates

A. ROY (1), N. Choudhary (1), V. D. Damsteegt (2), J. S. Hartung (3), R. H. Brlansky (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.; (2) USDA-ARS, Foreign Disease-Weed Science, Fort Detrick, MD, U.S.A.; (3) USDA-ARS, Beltsville, MD, U.S.A.
Phytopathology 100:S111

The most important viral disease of citrus is caused by *Citrus tristeza virus* (CTV). CTV infection often exists in field isolates as a complex of multiple genotypes. Aphid transmission is important for CTV dispersal. The complete 3′ terminal half sequences of the asymptomatic CTV isolate B192 and its single aphid transmitted (AT) sub-isolates were compared. Using genotype specific primers, the CTV-B192 source was identified as mixture of T30 and VT genotypes. However the T30 genotype was absent in 1st or 2nd level AT sub-isolates. Moreover, two AT sub-isolates contained additional T3 genotype along with VT in mixed infections. These two subisolates infected plants showed symptoms of severe vein clearing, vein corking in Mexican lime and stem pitting in sweet orange and grapefruit. The VT genotype was the minor genotype in the source isolate but was identified as the major and most transmissible genotype in the AT sub-isolates. Sequence analyses of p6, p23, p27, p33, p61 and p65 genes of CTV-B192 were homologous with asymptomatic isolate T30 while the sequence of p13, p18 and p25 genes were phylogenetically related to the New Zealand resistance breaking isolates than the T36 group isolates. The p20 sequence showed a 93% sequence identity with T36 isolates. The complete 3′ terminal half sequence derived from the source and AT sub-isolates clustered with asymptomatic T30 and symptomatic VT genotypic isolates, respectively, in the phylogenetic tree.

Endophytic bacteria in the inhibition of the germination of spores of *Ustilago scitaminea*

L. RUARO (1), S. R. de Souza (1), E. Daros (1), R. B. Fragoso (1)
(1) Universidade Federal do Parana, Curitiba, BRAZIL
Phytopathology 100:S111

Ustilago scitaminea, that cause the smut of the sugarcane if spreads and survives mainly through the teliospores. The use of isolated bacteria of the sugarcane, with antagonistic potential to inhibit the germination of these espores can consist in option in the reduction of the diseases. Four isolated bacterial ones: *Herbaspirillumn seropedicae* (cepa SmR1), *Azospirillum brasiliense* (cepas: AbV5, AbV6, SF0) were evaluated in the inhibition of germination of teliospores. Blades with paraffin rings were used contemning the suspension with teliospores of the pathogen and suspension of the antagonist, kept in B.O.D. at 28°C. The percentage of germination of the teliospores was evaluated 4 h after inoculation. The average values of the inhibition percentage were calculated in relation the witness. The best ones resulted had been presented by *Azospirillum brasiliense* lineage SF0 and *Herbaspirillum seropedicae* SmR1 lineage, which 97 and 96% of the germination of the teliósporos had inhibited respectively. *Azospirillum brasiliense* lineages: AbV5, AbV6 had not differed statistical from the witness without application of bacteria. It is concluded that these organisms present potential in the reduction of the diseases, having to be tested in field.

Soybean cyst nematode infects roots of sugar beet

K. RUDOLPH (1), M. D. Bolton (2), B. D. Nelson (3)
(1) Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.; (2) USDA-ARS Sugar Beet Unit, Fargo, ND, U.S.A.; (3) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S111

Soybean cyst nematode (SCN; *Heterodera glycines*) is the most important pest of soybean in the world. With the increase of soybean production in the Red River Valley of North Dakota and Minnesota over the past decades, SCN has become a growing threat to local soybean production. The sugar beet cyst nematode (*Heterodera schachtii*), a devastating pathogen of sugar beet and a close relative to SCN, is currently not found in the Red River Valley. Because SCN is closely related to *H. schachtii*, we initiated studies to determine whether SCN is able to infect sugar beet seedlings. Five cultivars of sugar beet were grown in soil infested with eggs of SCN HG 0 and roots were examined within two weeks. SCN readily penetrated into the cortex of sugar beet seedling roots, as determined by microscopy and SCN-specific PCR primers. Studies are currently underway to ascertain whether infection by SCN increases disease susceptibility of sugar beet seedlings to common soil borne pathogens in the Red River Valley.

Characterization of *Pythium* and *Fusarium* species associated with soybean seeds and seedlings

J. Rupe (1), C. Rothrock (1), M. AVANZATO (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S111

The importance of *Pythium* and *Fusarium* spp. on soybean has received considerable attention but little is known about the spatial and temporal dynamics of *Pythium* and *Fusarium* spp. on the scale of individual seed/roots. The objective of this research was a) to characterize *Pythium* and *Fusarium* spp. associated with soybean seeds/seedlings, and b) to determine pathogenicity of both pathogens on soybean seeds/seedlings. Field soil was collected from two Arkansas locations during 2007 and 2008. Growth chamber experiments were set up at the average planting temperatures for April (21°C), May (25°C) and June (28°C). Four, 12, 24, 48 and 72 h after sowing and at the vegetative stages, Ve, Vc and V1, soybean seeds/seedlings were collected and plated on amended water agar medium to recover fungal/oomycete flora. Isolates of *Pythium* and *Fusarium* spp. were identified to species by morphological and molecular techniques and evaluated initially with an *in vitro* pathogenicity seed assay. Nine *Pythium* species, *P. sylvaticum*, *P. irregulare*, *P. ultimum*, *P. spinosum*, *P. mamillatum*, *P. dissotocum*, *P. accanthicum*, *P. attrantheridium*, and *P. logandrum* and five *Fusarium* species, *F. oxysporum*, *F. equiseti*, *F. tricinctum*, *F. solani* and *F. graminearum* were recovered from soybean seeds and seedlings. *P. sylvaticum*, *P. irregulare*, *P. ultimum*, *P. spinosum*, *P. mamillatum*, and *P. dissotocum*; and *F. oxysporum*, *F. equiseti*, and *F. tricinctum* were pathogenic to varying degrees on soybean seeds.

First report of blue stain fungi associated with decline of pine trees in Lebanon

A. T. SAAD (1), L. Hanna (1), M. Temsah (2)
(1) American University of Beirut, Beirut, LEBANON; (2) Beirut, LEBANON
Phytopathology 100:S111

Pine forests, mainly *Pinus pinea*, *P. halepensis* and *P. brutia* cover an area of about 17000 ha on the sandy western slopes of the Lebanese range of mountains and a few coastal areas. Lebanon is a small country on the Eastern side of the Mediterranean Sea. These pine forests are valued for their economic returns from nut and wood products, as well as recreational and touristic sites. Recently, a new disease appeared to invade *Pinus pinea* trees resulting in their quick decline and death within few years as brought to our attention by the National Forestry Service. Affected trees in Beirut on the coastal area and the mountainous Metn County showed chlorosis and wilting symptoms with galleries of bark beetles in which fungal growth was observed. Cut sapwood was blue stained. Isolations from affected trees in Beirut revealed the occurrence of *Ophiostoma* teleomorph with long necked perithecia and the *Sporothrix* anamorph. Isolates from samples collected from *P. pinea* trees at five different locations in the Metn County had *Ophiostoma* teleomorphs with perithecia lacking necks and all with *Leptographium* anamorphs. Studies on characterization, identification and speciation of the *Ophiostoma* isolates will be reported. This is the first report of the blue stain fungi on pines trees in Lebanon.

Survival of *Erwinia tracheiphila* on muskmelon (*Cucumis melo*) leaves during wetness periods

E. SAALAU ROJAS (1), A. Owens (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S112

Erwinia tracheiphila causes bacterial wilt of cucurbits. This bacterium is vectored by cucumber beetles (*Acalymma vittatum* and *Diabrotica undecipunctata howardi*). Transmission occurs through contact of viable *E. tracheiphila* infested beetle frass and fresh feeding wounds. The ability of *E. tracheiphila* to survive on leaves as an epiphyte is currently unknown. To determine the survival of *E. tracheiphila* on leaves, muskmelon plants were grown at 27°C with 14 h-photoperiod in growth chambers. At first true leaf stage, plants were spray-inoculated with a 1×10^7 CFU/ml suspension of *E. tracheiphila* until runoff using a hand-triggered sprayer. Inoculated plants were maintained at 100% RH and 26°C in a dew chamber for the duration of the experiment. Populations of *E. tracheiphila* were determined by leaf washings and dilution plating at 0, 12, 24, 36, and 48 hours after inoculation. On average, population size decreased from 10^6 CFU/g of fresh weight at 0 hours at inoculation to 10^4 CFU/g fresh weight 48 hours after inoculation. The results suggest that epiphytic survival of this pathogen on muskmelon may have an important role in disease transmission under favorable weather conditions.

Efficacy of extended-duration row covers in suppressing bacterial wilt on muskmelon (*Cucumis melo*) in Iowa

E. SAALAU ROJAS (1), J. C. Batzer (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S112

Bacterial wilt (pathogen: *Erwinia tracheiphila*) causes major losses on muskmelon in the U.S. By delaying removal of spunbond row covers until 10 days after anthesis, plants are protected from cucumber beetles that vector *E. tracheiphila*. Six field trials in Iowa during 2007- 2009 assessed the efficacy of this strategy for suppressing bacterial wilt. Treatments included 1) no row cover, 2) row covers removed at anthesis, 3) row covers removed 10 days after anthesis, with ends opened at anthesis, and 4) row covers removed 10 days after anthesis, with bumble bee boxes inserted under row covers at anthesis. In two trials, delaying removal of row covers until 10 days after anthesis provided durable protection from bacterial wilt (less than 10% wilted plants compared to 35–75% for treatments 1 and 2). When transplanting was delayed by one month, all row cover treatments provided protection from bacterial wilt (less than 10% incidence vs. 60% for treatment 1). In 2009 field trials, no wilt occurred in any treatment, possibly due to low populations of cucumber beetles. The results suggest that the protective effect of delayed-removal row covers is impacted by transplanting date and disease pressure.

Assessing genetic diversity of *Erwinia tracheiphila* strains isolated from different cucurbit hosts using rep-PCR

E. SAALAU ROJAS (1), J. C. Batzer (1), M. L. Gleason (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S112

In 2008 and 2009, *E. tracheiphila* strains were isolated from cucurbit plants exhibiting bacterial wilt symptoms. Colonies that matched the morphology of *E. tracheiphila* were confirmed by PCR using *E. tracheiphila*-specific primers (ET1-2 or ETC1-2). Purified genomic DNA of 15 strains isolated from muskmelon (*Cucumis melo*), cucumber (*Cucumis sativus*), and squash (*Cucurbita pepo*) from eight U.S. states was amplified using the rep-primer sets ERIC1-2 and BOXA1R. DNA band patterns were observed after agarose gel electrophoresis. The patterns obtained from Cucurbita hosts were distinct from those of *Cucumis* spp. Strain profiles of *C. melo* and *C. sativus* showed very similar banding patterns across species and states. This study is the first to report genetic diversity among *E. tracheiphila* strains. The results suggest that *E. tracheiphila* strains could be specific for cucurbit host genera.

Genomic sequences and simultaneous detection of two cryptic viruses from pepper

S. Sabanadzovic (1), R. A. VALVERDE (2)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (2) Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, Baton Rouge, LA, U.S.A.
Phytopathology 100:S112

Pepper (Capsicum annuum L.) has been reported to contain a range of endogenous dsRNA molecules likely representing the genomes of distinct cryptoviruses. Indeed, partial sequences of Pepper cryptic virus 1 (PCV-1) have recently been generated from Jalapeno M pepper. In this work we have concluded the study on the PCV-1 genome and completely sequenced the genome of another cryptovirus designated as Pepper cryptic virus 2 (PCV-2). The two viruses could be distinguished by dsRNA pattern and shared limited identical amino acid content in both genomic segments. Interestingly, in the genomic molecule encoding the putative RNA-dependent RNA polymerase, they shared a higher level of common amino acids with cryptoviruses reported from other crops (i.e. Raphanus sativus cryptic virus 3, Black raspberry cryptic virus) than to each other. Two sets of virus-specific primers were successfully applied for the simultaneous and discriminative detection of these two viruses in various pepper germplasm.

A new ilarvirus from subgroup 1 infects ligustrum

S. SABANADZOVIC (1), I. E. Tzanetakis (2)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (2) Department of Plant Pathology, Division of Agriculture, University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S112

A mechanically transmissible virus was repeatedly isolated from young leaves of ligustrum specimens displaying virus-like symptomatology collected in Mississippi. Electron microscope observations of leaf dip preparations revealed the presence of quasi-isometric virions resembling the members of the family *Bromoviridae*, which was in agreement with the dsRNA patterns obtained from the same plants. Shotgun cloning followed by sequence comparisons and phylogenetic analyses indicate that this virus is an as yet undescribed member of Subgroup I in the genus *Ilarvirus* closely related to Strawberry necrotic shock and Blackberry chlorotic ringspot viruses. This virus, provisionally named Ligustrum line pattern virus (LLPV) has been found in several ligustrum specimens showing severe line pattern and ring spot symptoms indicating its possible involvement in the etiology of the disease.

Viruses of plants in the Great Smoky Mountains National Park

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S112

The majority of known plant viruses have been described from cultivated plants and/or agronomic weeds. Despite the fact that natural, non-agronomic ecosystems represent an excellent and unexplored substrate for study on virus ecology, taxonomy and evolution they remain understudied. For this reason, in 2006/2007 we initiated a study on phytoviruses in the Great Smoky Mountains National Park (GSMNP) within the framework of on-going All Taxa Biodiversity Inventory (ATBI) activities. In a few years, we successfully identified a number of phytoviruses, most of which represent as yet undescribed species. Among them, several viruses belong to genera currently represented by just one or few members (i.e. gen. *Petuvirus*, gen. *Enamovirus*, gen. *Oryzavirus*), or were detected also in some cultivated crops (i.e. blackberries, grapevines) indicating their possible economic importance.

First virus infecting Kudzu in U.S.A.

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1), W. F. Moore (1), T. W. Allen (2), R. C. Stephenson (1), A. Lawrence (3)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.; (2) Delta Research and Extension Center, Mississippi State University, Stoneville, MS, U.S.A.; (3) Electron Microscope Center, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S112

Kudzu (*Pueraria montana* var. lobata), a plant native to the Southeastern Asia, was originally introduced into the southeastern United States for forage and erosion containment. Adapting extremely well to the local climate, kudzu has since become a major noxious weed covering millions of acres. Curiously, despite intensive study on methods of its control, no viral diseases have been reported on kudzu in the U.S. Virus-like symptoms consisting of mottling and ring spots were observed in October 2009 on kudzu leaves in Northeastern Mississippi. Laboratory analyses showed the presence of high molecular weight dsRNA molecules in infected tissue, but not in the controls, indicating virus involvement in the disease. Electron microscope observations of partially purified extracts showed the presence of flexuous virions. Partial sequencing of the genome showed that the virus from kudzu is a potyvirus belonging to the Bean common virus subgroup. This is the first virus reported to infect kudzu in the U.S.

Another marafivirus infecting blackberries

S. SABANADZOVIC (1), N. Abou Ghanem-Sabanadzovic (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S112

In the course of a study on viruses of cultivated blackberries, a symptomatic specimen BB-R-1, collected from a backyard in Northern Mississippi, resulted positive in RT-PCR for tymo/marafiviruses using a general primer set. However, no products were observed in RT-PCR with specific primers for the

recently reported Blackberry virus S (the only virus belonging to the family *Tymoviridae* known to infect small fruits), which prompted further work on this virus. Complete sequencing showed that the genome of this virus shares characteristics with members of the genus *Marafivirus*. Pairwise comparisons of the complete polyprotein encoded by this virus with related viruses showed limited levels of amino acid identities indicating that this virus is likely a new species in this taxon. This virus was found in several other locations in Mississippi, suggesting that it may be common in blackberry production fields in the Southern United States. Its effect on the host is yet to be understood.

ITS-RFLP as a criterion to study identification of *Rhizoctonia solani*

M. SAFFARIAN ABBAS ZADEH (1), S. Soltani Nejad (2), R. Farrokhi Nejad (1), B. Mahmoudi (3)
(1) Department of Plant Protection, College of Agriculture, Shahid Chamran University-Ahvaz, Ahvaz, IRAN; (2) Ahvaz, IRAN; (3) Sugar Beet Seed Institute, Karaj, IRAN
Phytopathology 100:S113

Rhizoctonia solani is a world wide soil-borne pathogenic fungus showing the tremendous variation in characteristics such as morphology and host specificity. Traditional identification of *R. solani* based on anastomosis behavior did not provide adequate evidence for identification of different AGs and sub groups. In this study, for finding valid and rapid method for identification, hyphal anastomosis reaction and ITS-RFLP of isolates representing *R. solani* recovered from sugar beet root rot and potato tuber with black scurf were compared. Entirely, three anastomosis groups AG3, AG4 and AG5 recovered from potato and four anastomosis groups AG2, AG3, AG4 and AG5 and 8 unknown isolates (from dry rot symptom) obtained from sugar beet. In molecular studies, a DNA fragment of 700-750 bp in size was amplified from rDNA preparations of all isolates with the ITS5&4. The amplified products of DNA for all the isolates digested with the enzymes *Taq*1, *Bsur*1, *EcoR*1, and *Tru9*1. ITS-RFLP showed that isolates of different AGs generated very distinct patterns and were separated based on AGs as well as sub AGs. For instance, *Tru9*1 was sufficient to discriminate among different AGs and subgroups. Also, in the present study, the polymorphism existing among different isolates permitted the characterization of dry rot isolates for the first time. According to our results, Dry rot isolates are probably placed in AG1. Generally, it seems that ITS-RFLP technique is a precise method for study of identification of *R. solani* isolates.

Resistance to *Maize streak virus* in testcrosses of early generation lines of maize

M. T. SALAUDEEN (1), A. Menkir (2), G. I. Atiri (3), S. Hearne (2), P. Lava Kumar (2)
(1) International Institute of Tropical Agriculture (IITA) and Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, NIGERIA; (2) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA; (3) Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, NIGERIA
Phytopathology 100:S113

Maize streak disease caused by *Maize streak virus* (MSV, genus *Mastrevirus*) is a major disease of maize (*Zea mays* L.) unique to African continent. MSV is mainly controlled through host plant resistance. In this study, 250 testcrosses of S2 lines of maize were evaluated for MSV resistance under field conditions during 2009 wet season in Nigeria. Plants were inoculated at seedling stage using experimentally reared viruliferous leafhoppers, *Cicadulina triangula*. There was no immunity to MSV, however, substantial differences were observed in host response to the virus among testcross lines ($P < 0.01$). Six testcrosses were highly resistant, 71 were resistant, 146 moderately resistant, 27 were susceptible. Disease severity was negatively correlated with grain weight (-0.028), grain yield (-0.030), ear number (-0.214), kernel weight (-0.155), and kernel number (-0.023). MSV resistance in most genotypes was found to be recovery type, i.e., plants were infected and showed severe symptoms at early stage and reduction in symptoms in subsequently emerged leaves. MSV detection by enzyme-linked immunosorbent assay indicated high virus concentration in symptomatic leaves and low or undetectable levels in moderate or asymptomatic leaves suggesting positive correlation between symptoms and virus titer in plants. Inbred parents of the test crosses will be exploited to identify markers linked to MSV resistance using single nucleotide polymorphism (SNP) markers.

Relationship between grain yield and Fusarium Head Blight in soft red winter wheat as influenced by cultivar resistance

J. D. SALGADO (1), M. W. Wallhead (1), L. V. Madden (1), P. A. Paul (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S113

Fusarium Head Blight (FHB) of wheat is predominantly caused by *Fusarium graminearum* (teleomorph: *Gibberella zeae*) in North America. FHB affects wheat by reducing grain fill and kernel size, leading to low test weight and yield loss. However, it is unclear how yield loss is influenced by relative susceptibility of the affected cultivar to FHB. Field plots of three soft red winter wheat cultivars with different levels of FHB resistance (Hopewell, Truman and Cooper, moderately susceptible, moderately resistant, and susceptible to FHB, respectively) were planted and inoculated with *G. zeae/F. graminearum*, with the objective of characterizing the relationships between FHB and grain yield. Plots were spray-inoculated at anthesis (Feekes 10.5.1) with spore concentrations ranging from 0 to 150,000 spores/mL. FHB intensity was estimated at soft dough and yield determined following harvest. Averaged across inoculation treatments, mean FHB index and grain yield ranged 1.49 to 24.64% and 5,552 to 6,311 Kg/ha for Cooper; 3.76 to 42.06% and 3,975 to 5,298 Kg/ha for Hopewell; and 0.67 to 9.61% and 5,473 to 5,907 Kg/ha for Truman. Based on regression slopes, yield reduction per unit increase in index varied among cultivars, being highest for moderately susceptible Hopewell, intermediate for moderately resistant Truman and lowest for susceptible Cooper, with estimated losses of 27.77, 23.91 and 20.26 Kg/ha per unit increase of disease index, respectively, for the three cultivars.

New and re-emerging rust diseases from Idaho and Oregon

R. SAMPANGI (1), M. C. Aime (2), K. Mohan (1), C. Shock (3)
(1) University of Idaho, Parma, ID, U.S.A.; (2) Louisiana State University, Baton Rouge, LA, U.S.A.; (3) Oregon State University, Ontario, OR, U.S.A.
Phytopathology 100:S113

New host–pathogen records contribute to vital information that aids in quick and accurate diagnosis of plant diseases. New pathogen reports help in establishing baseline data about pre-existing and emerging plant pathogens and the epidemiology of the diseases they cause, thus furthering the understanding of pathogen biology, host ranges, and the geographic range of pathogens. First reports contribute to data on disease occurrences and resulting host-fungus indices can serve as primary resources for information to plant disease diagnosticians, extension educators, plant health professionals, and regulatory officials. Rust diseases, caused by fungi in the basidiomycete order Pucciniales, are one of the most important agents of agricultural losses. Presented herein are examples of new and reemerging rust diseases on regional crops turf grass, forbs and forages from Idaho and Oregon, all noted between 2006–2009: *Puccinia graminis* Pers.:Pers on *Poa pratensis* L, *Puccinia similis* Ellis & Everh on *Artemisia tridentata* Nutt., *Puccinia jonesii* Peck on *Lomatium dissectum* (Nutt.) Mathias & Constance, *Puccinia sherardiana* Körn on *Sphaeralcea grossulariifolia* (Hook. & Arn.) Rydb and *Uromyces intricatus* Cooke on *Erigonum umbellatum* Torr.

Nematode and bacterial associates of the invasive Brown Garden Snail: *Helix aspersa*

K. SANCHEZ (1), C. Pagan (1), S. A. Nadler (1), E. P. Caswell-Chen (1)
(1) University of California, Davis, CA, U.S.A.
Phytopathology 100:S113

Helix aspersa (Brown Garden Snail) is an invasive terrestrial mollusk. It is a pest of plants and can damage young seedlings and foliage. We collected 350 snails in California (San Francisco, Sacramento, Davis, Woodland, San Jose, and Tulare) to identify associated nematodes and bacteria, and to determine snail tissues where nematodes and bacteria occur. Snails were dissected, and nematodes and bacteria were recovered from surface rinsate, the foot muscle, shell, digestive gland, stomach, heart, mantle, and feces yielding 500 individual nematodes. Nematodes and bacterial isolates were subject to PCR amplification using primers for ITS, 16S, 28S, 18S, and rpoβ. Nematodes were recovered from ca. 91% of snails and included *Caenorhabditis elegans* (in 60% of snails), *Rhabditis terricola* (32%), *Aphelenchoides fragariae* (44%), *Xiphinema index* (24%), *Heterodera* spp. (28%), and *Aphelenchus avenae* (45%). There were 25 distinct bacterial colonies isolated from organ tissues, with *Serratia proteamaculans*, *Klebsiella terrigena*, and *Stenotrophomonas maltophilia* recovered, and five bacteria isolated from snail slime, including *Psuedomonas putida* and *Sphingobacterium kitahinoshimense*. These associations establish the brown garden snail's role as an important phoretic host for plant pathogens, and lead us to consider the possible use of snails as sentinels for the detection of pathogens in the environment.

Effect of fungicide dip treatments on pink root disease and yield of transplanted sweet onions in Georgia

F. SANDERS, JR. (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S113

Pink root disease of onion caused by the fungus, *Phoma terrestris*, is a common soil borne disease in the onion growing region of Georgia and growers experience yield reductions due to pink root every year. The fumigant Vapam (metam sodium) is used to control pink root in onion seed beds however, this treatment is too expensive to apply to production fields. In this investigation, dip treatments with the fungicides Endura (boscalid), Topsin

(thiophanate methyl) Switch (cyprodinil + fludioxinil) were evaluated for pink root suppression. Trials were conducted in two consecutive growing seasons at the Vidalia onion and Vegetable Research and Educational Center in Lyons, Georgia. The experimental design for both trials was a 2 × 2 factorial with variety and fungicide treatment as factors with four replications. Onion transplants were treated with fungicide dips at plant and compared to foliar fungicide sprays and an untreated control. Pink root incidence was taken prior to harvest in both trials by examining the roots of 10 plants per plot for disease symptoms, and yields were taken. Endura dip treatments reduced pink root incidence and increased yield when compared to the untreated control, and there was no difference in yield between plots treated with Endura dips and plots treated with three foliar sprays of Pristine (boscalid + pyraclostrobin) in both trials. Transplant dip treatments with Endura have the potential to increase onion yields and provide suppression of pink root.

Etiology of pod rot of Valencia peanut in New Mexico

S. SANOGO (1), N. Puppala (2)
(1) New Mexico State University, Las Cruces, NM, U.S.A.; (2) Clovis, NM, U.S.A.
Phytopathology 100:S114

Soilborne pathogens typically causing pod rot on peanut (Arachis hypogaea) include *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium* species. Although pod rot is known to occur in New Mexico, no etiological study has been conducted on this disease. In 2005 and 2006, Valencia peanut fields were surveyed in eastern New Mexico in order to characterize mycelial microorganisms associated with pod rot. Peanut plants were collected and processed for isolation of microorganisms by plating seeds, pieces of shell, root, and stem on acidified potato dextrose agar. In both years, a diverse group of mycelial microorganisms were recovered from all plant part tissues, with *Rhizoctonia solani* as the most predominantly isolated microorganism. In 2005, *R. solani* was found in all fields with frequency of isolation varying from approximately 7% to 43% across all plant part types. Frequency of isolation was approximately 12 to 32% for root, 7 to 43% for seed, 8 to 38% for shell, and 8 to 27% for stem. In 2006, *R. solani* was found in all fields with frequency of isolation varying from approximately 5% to 45% across all plant part types. Frequency of isolation was approximately 32 to 45% for root, 5 to 22% for seed, 25 to 28% for shell, and 20 to 27% for stem. In controlled environment studies, all isolates of *R. solani* from different plant parts were shown to be pathogenic to Valencia peanut. Based on the findings from this study, pod rot management in New Mexico should include steps to target *R. solani*.

***Colletotrichum capsici* and *Colletotrichum coccodes*: Predominant causal agents of anthracnose of chile pepper in New Mexico**

S. SANOGO (1)
(1) New Mexico State University, Las Cruces, NM, U.S.A.
Phytopathology 100:S114

The occurrence of anthracnose of chile pepper (*Capsicum annuum*) has been reported in various extension publications in New Mexico. However, the causal agents of this disease have not been identified. Symptoms are typically expressed as depressed sunken lesions on ripe fruit. In 2008 and 2009, chile pepper fruit with symptoms of anthracnose were collected from several fields in southern New Mexico. Acervuli were observed on calyces and on surface of lesions. Chile pepper plants with blighted stems covered with acervuli were found. Microscopic observations of acervuli revealed the presence of two major types of conidia characteristic of *Colletotrichum capsici* and *C. coccodes*. Fruit and stem tissue plated on acidified potato dextrose agar yielded predominantly colonies of *C. capsici* and *C. coccodes*. Both species were isolated from the same fruit and stems of several samples. On potato dextrose agar, *C. coccodes* produced abundant sclerotia whereas *C. capsici* produced none. Radial growth of two isolates, one of each species, was compared at 25 and 30°C. Whereas radial growth of both isolates was similar at 25°C, radial growth of *C. capsici* isolate was over 30% greater than that of *C. coccodes* isolate at 30°C. Both isolates caused typical anthracnose symptoms on ripe chile pepper fruit inoculated with conidia of each isolate. This etiological study indicates that at least two species of Colletotrichum are primarily associated with anthracnose of chile pepper in New Mexico.

Field evaluation and genetic characterization of a quantitative trait locus conferring resistance to Southern leaf blight

J. SANTA-CRUZ (1), A. Belcher (2), J. Zwonitzer (3), C. Arellano (4), M. Krakowsky (5), P. Balint-Kurti (6)
(1) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) Research and Extension Center, University of Idaho, Aberdeen, ID, U.S.A.; (3) Dow Agrosciences, Huxley, IA, U.S.A.; (4) Dept. of Statistics, North Carolina State University, Raleigh, NC, U.S.A.; (5) Dept. of Crop Science, North Carolina State University, Raleigh, NC, U.S.A.; (6) Dept. of Plant Pathology, North Carolina State University, USDA-ARS, Plant Science Research Unit, Raleigh, NC, U.S.A.
Phytopathology 100:S114

Southern leaf blight (SLB) caused by the fungal pathogen *Cochliobolus heterostrophus* (anamorph = *Bipolaris maydis*), is a common disease of maize in southeastern U.S., as well as many hot and humid tropical and subtropical areas in the world. Most of the disease resistance used in maize is quantitative in nature; however, quantitative disease resistance remains poorly understood. To investigate quantitative resistance to SLB, we used the highly resistant inbred maize line NC292. This line is derived from crossing NC250, an elite source of SLB resistance, to the highly susceptible line B73 followed by three further backcrosses to B73 and several rounds of selfing. At each stage in this process the plants were selected for SLB resistance. Using genome-wide marker analysis of NC292, we detected 12 NC250-specific introgressions. Furthermore, 9 disease QTLs associated with SLB resistance were mapped on a related population, from which 4 colocalized with NC250-specific introgressions. We identified a strong QTL for SLB resistance at the tip of the short arm of chromosome 6 of maize which colocalized with a NC250 introgression in NC292, namely introgression 6A. Specific objectives of this research include fine-mapping introgression 6A, cloning of the gene that accounts for this effect, and evaluating yield and fitness effects of this introgression under both high and low disease pressure for possible future use of this resistance. Preliminary growth chamber phenotyping experiments showed that introgression 6A segregates as a single recessive resistance gene, and can be scored in growth chamber experiments on a single plant basis. Over 168 F2 individuals and over 300 F2:3 families were phenotyped, and genotyped with SNPs markers in order to narrow down the region of interest (< 1.5Mb). Currently, candidate genes are being analyzed. To evaluate fitness and yield, we have developed isohybrid pairs by crossing B73 with/without introgression 6A to several inbred lines (testers). We have found significant differences between the treatments in some pedigrees. Summer yield experiments are being designed to study the influence of the presence or absence of 6A introgression on agronomic traits and disease.

Detection of reniform nematode by conventional and real-time PCR

R. J. SAYLER (1), T. L. Kirkpatrick (1), R. T. Robbins (1), R. D. Cartwright (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S114

Reniform nematode *Rotylenchulus reniformis* is a recent introduction into the continental U.S. This plant parasite has a wide host range including economically important crops such as cotton and soybeans corn. Worldwide, the reniform nematode typically occupies tropical and subtropical environments, and appears to be restricted to the southern U.S. from Texas east to the Atlantic ocean and as far north as the Missouri Bootheel. The goal of this project is to optimize protocols for both conventional and real-time PCR that identify the reniform nematode on the basis of the sequence of the internal transcribed spacer (ITS) region of this species. A PCR-based detection and quantification method for this nematode may provide a more rapid and less labor-intensive alternative to visual identification for diagnosticians and extension specialists, which in turn should yield more timely management recommendations for growers. These primers have successfully amplified a 240 base pair fragment of the ITS region from this nematode.

Improved extraction of DNA of *Ca.* Liberibacter species from plants and cultivated cells using pressure cycling technology (PCT)

N. Schaad (1), A. SECHLER (2), A. Marques (3), N. Lawrence (4), R. Schumacher (4)
(1) USDA ARS NAA FDWSRU, Ft. Detrick, MD, U.S.A.; (2) FDWSRU, USDA-ARS, Ft. Detrick, MD, U.S.A.; (3) EMBRAPA, Brasilia, BRAZIL; (4) Pressure BioSciences Inc, South Easton, MA, U.S.A.
Phytopathology 100:S114

Huanglongbing, one of the most destructive diseases of citrus, is caused by three species of *Ca*. Liberibacter. Diagnosis of the disease is reliant on real-time PCR (RT-PCR). Detection of the pathogen is complicated, especially from small survey samples, because of low titer and uneven distribution of the bacterium throughout the infected plant and the complex nature of the plant tissue from which it is extracted. Cultured cells of *Ca*. Liberibacter species are also difficult to disrupt for efficient DNA extraction. Pressure cycling technology (PCT) is a dynamic technique that can be used for the highly-efficient extraction of protein and nucleic acids from simple and complex samples. Here we compare DNA extraction methods on the three known citrus species of *Ca*. Liberibacter from infected plants and cultured cells using PCT with the PCT Shredder™ and NEP 2320 Barocycler (Pressure BioScience, South Easton, MA) and commercially available DNA extraction kits. Combinations of these techniques and methods were tested and the resulting samples were used with RT-PCR. Preliminary results from pressure cycling

together with The PCT Shredder™ found an increase in RT-PCR positive samples of *Ca*. Liberibacter species by 12–42% from infected plants over other methods and an increase in DNA yield from cultured cells.

Moderate temperature fluctuations rapidly reduce viability of *Ralstonia solanacearum* Race 3 biovar 2 in infected geranium, tomato, and potato

J. M. Scherf (1), A. Milling (1), C. ALLEN (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S115

Ralstonia solanacearum Race 3 biovar 2 (R3bv2) causes bacterial wilt of potato, tomato, and geranium plants in the highland tropics and temperate zones where more typical tropical *R. solanacearum* strains don't cause disease. R3bv2 is a high-concern quarantine pathogen in Europe and Canada and a U.S. Select Agent pathogen because it could threaten the potato industry if it became established in cool temperate zones. Previous experiments revealed that R3bv2 did not survive as well as subtropical US Race 1 (R1bv1) strains in water at 4°C, but that R3bv2 survived longer than R1bv1 in potato tubers at 4°C. To better understand this key epidemiological trait, we measured survival of R3bv2 and R1bv1 in infected tomato and geranium plants, and in infected potato tubers following typical temperate winter temperature cycles of 2 days at –5°C followed by 2 days at +10°C. Population sizes of both strains were initially above 10e7 cfu/gm, but they declined rapidly under these conditions in all three plant hosts. No culturable *R. solanacearum* cells could be detected after 6 to 7 cycles. The temperature fluctuations are critical for loss of bacterial viability since at a constant temperature of –20°C, large populations of both tested strains survived in infected plant tissue for at least 6 months. These results suggest that even when sheltered in infected plant tissue, R3bv2 is unlikely to survive the temperature fluctuations that commonly occur during a northern U.S. winter.

Investigating the ironwood tree (*Casuarina equisetifolia*) decline on Guam using applied multinomial modeling

K. A. SCHLUB (1), B. D. Marx (1), Z. Mersha (2), R. L. Schlub (2)
(1) Department of Experimental Statistics, Louisiana State University, Baton Rouge, LA, U.S.A.; (2) Guam Cooperative Extension Service, University of Guam, Mangilao, GUAM
Phytopathology 100:S115

The ironwood tree (*Casuarina equisetifolia*), a protector of coastlines of the sub-tropical and tropical western Pacific, is in decline on the small island of Guam, where aggressive data collection and efforts to mitigate the problem are underway. For each sampled tree, the level of decline was measured on an ordinal scale consisting of five categories, ranging from healthy to near dead. Several predictors were also measured including tree diameter, fire damage, typhoon damage, presence or absence of termites, presence or absence of basidiocarps, and various geographical or cultural factors. The five decline response levels can be viewed as categories of a multinomial distribution, where the multinomial probability profile depends on the levels of these various predictors. Such data structure is well suited to a proportional odds model, thereby leading to odds ratios, involving cumulative probabilities which can be estimated and summarized using information from the predictor coefficient. Various modeling techniques were applied to address data set issues: reduced logistic models, spatial relationships of residuals using latitude and longitude coordinates, and correlation structure induced by the fact that trees were sampled in clusters at various sites. Among our findings, factors related to ironwood decline were found to be latitude, basidiocarps, termites, and level of human management.

Culture-independent examination of microbial community shifts associated with replant disease of almond

L. S. SCHMIDT (1), R. G. Bhat (2), G. T. Browne (1)
(1) USDA ARS CPGRU, Davis, CA, U.S.A.; (2) University of Californa, Davis, CA, U.S.A.
Phytopathology 100:S115

Growth and productivity of successive almond and stone fruit plantings can be severely suppressed by Prunus replant disease (PRD). PRD occurs in absence of plant parasitic nematodes, is associated with poor root health, and is prevented by soil fumigation, but its etiology is poorly resolved. We used culture-independent (CI) methods to examine microbial communities associated with PRD in two California almond orchards [Sacramento Valley (SV), San Joaquin Valley (SJV)]. Total DNA was extracted from roots (≤1 mm diam.) of healthy and PRD-affected trees and used for PCR amplification of rDNA from bacteria, stramenopiles, and fungi. The amplicons were cloned, sequenced, and grouped into operational taxonomic units (OTUs). At the SJV site, redundancy analysis (RDA) discriminated shifts in bacterial, fungal, and stramenopile populations associated with PRD (P = 0.02, 0.05, and 0.06, respectively). Similar trends were observed at the SV site, but the ordinations were not significant (P = 0.13 to 0.30). At both sites, *Cylindrocarpon destructans* and *Phaeonectriella lignicola* were found predominately in PRD-affected roots. Previous culture-based (CB) examinations of the SV and SJV samples (RDA of morphological OTUs) associated *Cylindrocarpon* spp., *Fusarium* spp., other fungi, and *Pythium* spp. with PRD at both locations (P = 0.002 for ordinations). Both in healthy and diseased roots, some organisms detected by CI methods were not discriminated by CD methods, and vice versa. Therefore, both methods appear essential to resolve PRD etiology.

Efficacy of phosphonate treatments against Sudden Oak Death in Tanoaks

D. SCHMIDT (1), M. Garbelotto (1)
(1) University of California Berkeley, Berkeley, CA, U.S.A.
Phytopathology 100:S115

Pytophthora ramorum, the causal agent of Sudden Oak Death (SOD), has killed hundreds of thousands of trees in California and Oregon. Tanoaks (*Lithocarpus densiflorus*) are both stem and foliar hosts and, as such, die from SOD and help spread the disease. Phosphonate treatments are routinely used in agricultural and orchard crops affected by *Phytophthora* diseases. We have developed a detached-leaf bioassay for studying the effectiveness of phosphonate treatments for SOD in tanoaks. The assay involves infecting the petioles of tanoak leaves with agar plugs of *P. ramorum* in culture. SOD infection is analyzed by examining the spread of *P. ramorum* down the midrib of the leaf. This assay has shown that tanoaks in wildland settings, treated with phosphonates, are resistant to SOD infection. In addition, we are maintaining long-term studies of tanoaks treated with phosphonates in SOD infected forest areas. Paired 20mx20m treatment and control plots were established near existing SOD infections. The trees were evaluated for disease symptoms and general health prior to the initial treatment and each subsequent year. The results show that phosphonate treatments are effective at slowing and preventing the spread of the disease in the treated areas. Treatments at the leading edge of SOD infected areas were less effective, confirming that phosphonate treatments are significantly more effective as preventative rather than curative treatments.

Factors affecting the development of the green stem malady in soybean

R. W. SCHNEIDER (1), G. B. Padgett (2), D. J. Boquet (2), R. A. Valverde (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.; (2) Louisiana State University Agricultural Center, Winnsboro, LA, U.S.A.
Phytopathology 100:S115

The soybean disorder known as the "green stem malady" has recently become one of the primary constraints to soybean productivity in the Mid South. Symptoms may assume several guises, but the common diagnostic symptom is stems and often leaves remain green while the pods mature. This causes the stems to remain supple, and the thrasher on the harvester becomes congested with stems. A multi-disciplinary field study was conducted in 2008 and 2009 at three locations in Louisiana in an attempt to determine the causes of this disorder. The following factors were investigated in multifactorial experiments: cultivars, the fungicide pyraclostrobin (Headline), the herbicide glyphosate (Roundup), presence of specific viruses, and irrigation. Cultivars were the dominant statistical factor in symptom development. There was a significant interaction between cultivar and Headline such that symptom expression was more severe in sensitive cultivars following the application of the fungicide. Likewise there were significant interactions between Headline and irrigation, between Roundup and irrigation, and between cultivar and irrigation. There was no interaction between Headline and Roundup. None of the viruses appeared to be related to the disorder.

Phylogenetic analysis and population identification of the phyopathogen *Xylella fastidiosa* using *zot* and *gyrB* genes

H. L. SCHREIBER (1), J. M. Repshare (1), C. E. Skipper (1), L. D. Morano (2), B. R. Bextine (1)
(1) University of Texas at Tyler, Tyler, TX, U.S.A.; (2) University of Houston - Downtown, Houston, TX, U.S.A.
Phytopathology 100:S115

The phytopathogenic bacterium *Xylella fastidiosa* has been separated into four subspecies, *fastidiosa*, *sandyi*, *multiplex*, and *pauca*, through analysis of genetic sequence similarity. These subspecies cause disease in distinct plant hosts, including economically important crops, such as Pierce's Disease of grapevines, oleander leaf scorch, phony peach disease, and citrus variegated chlorosis. In this study, 40 Texas strains of *X. fastidiosa* subsp. *fastidiosa*, *sandyi*, and *multiplex* were classified using DNA sequence comparisons of orthologous *Zonula Occludens Toxin* (*zot*) and *gyrase B* (*gyrB*) genes. Previous phylogenetic analyses have used the highly conserved *gyrB* gene to categorize *X. fastidiosa* strains into subspecies. The *zot* gene in *X. fastidiosa* is homologous to *zot* genes in other gammaproteobacteria which produce the exotoxic protein Zot, and orthologous and paralogous *zot* genes have been identified in each subspecies of *X. fastidiosa*. Classification of *X. fastidiosa* strains using the *zot* gene produced accurate cladistical groupings into subspecies similar to those produced by analysis of the *gyrB* gene.

Additionally, *zot* genes displayed genetic differences between Texas and California strains of *X. fastidiosa*, establishing the *zot* gene as a target to differentiate *X. fastidiosa* populations. The *zot* gene offers a novel target for phylogenetic analysis, and may offer additional disease tracking and identification opportunities for disease management protocols.

Expression rate of the *Zonula Occludens Toxin* (*zot*) gene in two growth states and two media types of *Xylella fastidiosa*

H. L. SCHREIBER (1), C. E. Skipper (1), J. M. Repshare (1), L. D. Morano (2), B. R. Bextine (1)
(1) University of Texas at Tyler, Tyler, TX, U.S.A.; (2) University of Houston - Downtown, Houston, TX, U.S.A.
Phytopathology 100:S116

Xylella fastidiosa is the causal agent of many plant diseases, inclduing Pierce's Disease of grapevines, citrus variegated chlorosis in orange trees, and leaf scorch in almonds, alfalfa, oleander, and coffee. A detailed pathogenic mechanism has not been described, though a combination of factors leading to xylem occlusion seems likely. Recently the *Zonula Occludens Toxin* (Zot) was noted as a possible virulence factor in *X. fastidiosa*. Zot is an exotoxin produced by the prophage gene *zot* which is homologous to the *zot* gene found in other phytopathogens, such as *Xanthomonas campestris* and *Ralstonia solanacearum*. Many pathogenic bacteria, including *X. fastidiosa*, lose virulency after serial passages through axenic media as the expression of virulence genes is downregulated. Using quanititative, real-time polymerase chain reactions (qRT-PCR), this study quantified the expression rate of the *zot* gene relative to a conserved housekeeping gene *gyrase B* (*gyrB*) in newly harvested (NH) and serially passed (SP) cultures of X. fastidiosa grown in axenic media or media augmented with extracted xylem sap from *Vitis vinifera*. This comparison revealed significant differences in *zot* expression rate between NH and SP cultures of *X. fastidiosa* as well as differences between rates *zot* expression in *X. fastidiosa* grown in axenic media and *X. fastidiosa* cultured in augmented media. These results support the hypothesis that the Zot exotoxin is a virulence factor in *X. fastidiosa*.

Attempting to transmit citrus canker from diseased ripe grapefruit to healthy grapefruit saplings under field conditions

T. SCHUBERT (1), G. Bonn (1)
(1) Florida Dept. of Agriculture & Consumer Services, Gainesville, FL, U.S.A.
Phytopathology 100:S116

Xanthomonas citri subsp. *citri* (*Xcc*) causing citrus canker is an important foliar and fruit pathogen of citrus in the sub-tropical agricultural regions of the world. In order to meet quality standards and phytosanitary regulations, citrus fruit packed and shipped from Florida for consumption must be free of canker lesions. Recent changes to the regulations allow fruit from cankered groves to be shipped domestically, leaving open the possibility that fruit with lesions might slip by graders to enter into existing canker-free production areas. To determine whether lesions on ripe fruit could lead to canker symptoms on susceptible citrus we laid out field plots to attempt transmission of *Xcc* from infected commercially-packed grapefruit. Diseased fruit surfaces as well as surfaces of adjacent susceptible grapefruit saplings were assayed for *Xcc* periodically during the growing seasons of Florida. Dilution plating of fruit and leaf swabbing onto a semi-selective medium and bioassays in greenhouse-grown grapefruit saplings indicated the occasional detection of *Xcc* on the surface of infected fruit following a wetting period (dew, rain and overhead watering). However, *Xcc* did not move from the diseased fruit to the adjacent healthy saplings. The low number of viable canker bacteria associated with peel lesions in ripe grapefruit, especially during wet warm periods, suggests that the risk of canker transmission from them is exceptionally low.

Sequencing *Candidatus* Liberibacter asiaticus from cultivated cells

E. Schuenzel (1), J. Crow (2), A. Sechler (3), R. Kim (2), N. SCHAAD (3)
(1) USDA ARS NAA FDWSRU, Ft. Detrick, MD, U.S.A.; (2) National Center for Genome Resources, Santa Fe, NM, U.S.A.; (3) FDWSRU, USDA-ARS, Ft. Detrick, MD, U.S.A.
Phytopathology 100:S116

Using amplified DNA from cultured cells and Illumina Solexa second generation sequencing technology, we sequenced *L. asiaticus* strain China1 previously shown to be pathogenic. A collection of 21M trimmed paired-end 90mer reads was aligned to all published bacterial genomes at NCBI using GSnap. 2,220 reads aligned to the current reference for *L. asiaticus* CP001677.2, primarily to regions identified as rDNA. Alignment to chromosome 1 and the plasmid of *Ralstonia pickettii* was observed. Reads also aligned to the family Rhizobiaceae. Over 90 percent of reads were novel, not aligning to any bacterial genome at NCBI. A draft Phase 1 assembly was constructed (contigs unordered and unoriented) resulting in 481 contigs, indicating a genome size of 3.8M bp. These contigs demonstrate local similarity to bacterial sequences at NCBI, containing novel material and ORFs similar to known genes or conserved domains. Sequence-derived evidence was used to confirm the presence or absence of these contigs in 8 culture and 34 diseased tissue samples. The presence of Rhizobiaceae-like sequence in all samples confirmed the successful sequencing of the genome from cultured cells of Liberibacter. Ralstonia plasmid sequence was found in 25% of Asian samples, but not in North and South American samples. Unique sequences showed two patterns; sequence present in all samples suggesting bacterial chromosomal DNA and sequence present in a percentage of the samples suggesting plasmid DNA.

Host-derived RNAi targeted to a novel root-knot parasitism gene in tobacco

K. K. SCHWERI (1), G. Huang (2), B. Xue (1), M. G. Mitchum (3), T. J. Baum (4), R. S. Hussey (2), R. Lewis (1), E. L. Davis (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.; (3) University of Missouri, Columbia, MO, U.S.A.; (4) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S116

Root-knot nematodes (RKN), genus *Meloidogyne*, have multiple crop host species and cause severe economic losses worldwide. Proteins encoded by RKN parasitism genes are secreted into host plant root cells to form elaborate and essential feeding cells. Previous studies have shown that silencing the novel 16D10 RKN parasitism gene transcript using host-derived RNA interference (RNAi) makes *Arabidopsis thaliana* plants highly resistant to all four major RKN species. The 16D10-RNAi construct that was used in the above study was used to transform two haploid lines of *Nicotiana tabacum*, TN90, a burley tobacco, and Hicks, a flue-cured tobacco. Double-haploids were recovered through midvein tissue culture of mature leaves, and T1 progeny were produced through self-fertilization. Nematode infection assays of T1 plants have shown a significant reduction in the number of eggs produced by females of *M. arenaria* when compared with wild-type. No off-target effects of 16D10 RNAi have been observed in the regenerated tobacco lines. Attempts to correlate RNA expression with the severity of the nematode infection are underway.

Movement, germination and production of *Puccinia pelargonii-zonalis* urediniospores on greenhouse-grown geraniums

E. A. SCOCCO (1), J. Buck (1)
(1) University of Georgia, Griffin, GA, U.S.A.
Phytopathology 100:S116

Geranium rust caused by *Puccinia pelargonii-zonalis* can result in significant monetary losses to greenhouse operators. Inoculum production and movement of urediniospores throughout the greenhouse can affect management options. The purpose of this research was to determine urediniospore production per pustule over a 24 h period and to track airborne movement of inoculum in a greenhouse. Geraniums cv. Maverick Red in 3.8 L pots were spray-inoculated with 10^5 urediniospores/mL until leaf wetness and then bagged for 24 h to initiate infection. After 12–14 days, urediniospores were vacuumed-collected from lesions every 24 h for 3 days. Urediniospore samples were suspended in 0.05% Tween 20, enumerated by hemacytometer, and four 50 µl aliquots from each sample were placed on water agar. Germination was assessed after 24 h (21°C) at 200X magnification. Movement of urediniospores along a greenhouse bench was assessed using three 3.8 L geraniums cv. Maverick Red with sporulating lesions. Rotorods were placed 0.3, 0.9, 1.5 and 2.1 m away from the inoculated plants at pot height and glass slides coated with petroleum jelly were placed on the bench at 0.3 m intervals. Glass slides and rotorods were collected after 8 h and observed at 100X magnification. An average of 1580 urediniospores were produced per pustule every 24 h and germination ranged from 37–59%. After 8 h, movement of urediniospores was detected from infected plants up to 1.82 m at bench level and 2.1 m at pot height.

Biocontrol & functional properties of pseudomonads isolated from different ecological niches & diversity of *phlD* a key gene in the 2, 4-DAPG biosynthesis

J. SEKAR (1), V. R. Prabavathy (1), S. Nair (1)
(1) M.S. Swaminathan Research Foundation, Chennai, INDIA
Phytopathology 100:S116

2, 4-diacetylphloroglucinol (DAPG) is a broad-spectrum antibiotic exhibiting biocontrol activity against plant pathogens. 1500 strains isolated from the rhizosphere region of different ecosystems viz., agricultural fields, coastal, hills & mangroves were screened for pseudomonads using *rpoD* primer. 350 isolates amplifying 760bp amplicon were considered as pseudomonads positive. On screening further for *phlD* gene, 45 strains amplifying a 745bp amplicon were selected as DAPG positive. Of these 120 positive pseudomonads from the agriculture field have 23 DAPG positive isolates, from 42 pseudomonads strains 5 codes for DAPG from the mangrove and 152 pseudomonads 17 DAPG positives from the saline coastal ecosystems. All the DAPG positives were partially sequenced & analyzed for their genetic diversity. Antagonistic activity of pseudomonads were assayed against plant

pathogens resulted in 27% suppressing *M. grisea* growth and 35% inhibit the growth of *X. oryzae* & *R. solani*. Isolates negative for DAPG also exhibited antagonistic activity and also isolates positive for DAPG coding strains failed to exhibit antagonistic activity against the plant pathogens. In that 55% of strains having coding genes for HCN, 37% for chitinase production, 8% for quorum sensing, 33% for biofilm formation, 15% for phosphate solubilizing, 63% for protease & 46% cellulase producers. Functional properties of pseudomonads were comparatively good in agricultural ecosystem and the role of other metabolite coding genes is in progress.

RPG1-B derived resistance to *AvrB* expressing *Pseudomonas syringae* requires RIN4-like proteins in soybean

D. SELOTE (1), A. Kachroo (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S117

Soybean RPG1-B mediates species-specific resistance to AvrB expressing *Pseudomonas syringae*, similar to the non-orthologous RPM1 in Arabidopsis. RPM1-derived signaling is presumably induced upon AvrB-derived modification of the RPM1-interacting protein, RIN4. Similar to RPM1, RPG1-B does not directly interact with AvrB. However, RPG1-B associates with RIN4-like proteins from soybean. Unlike Arabidopsis, soybean contains at least four RIN4-like proteins (GmRIN4a-d), all of which bind AvrB. In contrast, GmRIN4b, c, and d bind RPG1-B, but GmRIN4a does not. Silencing either GmRIN4a or b abrogates RPG1-B-derived resistance to *P. syringae* expressing AvrB. Binding studies show that the various GmRIN4 isoforms interact with each other. The lack of functional redundancy amongst the GmRIN4 proteins and their abilities to interact with each other, suggests that these proteins might function as a heteromeric complex in mediating RPG1-B-derived resistance. The GmRIN4 proteins also participate in soybean basal defense, since silencing *GmRIN4a* or *b* enhances basal resistance to virulent strains of *P. syringae* and the oomycete *Phytophthtora sojae.*

Comparison of transient expression vectors for production of recombinant proteins in plants

K. H. SHAH (1), H. Bohlmann (1)
(1) Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, AUSTRIA
Phytopathology 100:S117

Production of recombinant proteins in plants is getting more and more importance not only in plants research field but also in the field of medicines. Transient expression vectors are efficient tools for this purpose. To date, a large number of such vectors have been constructed. Each of these is reported to be highly efficient, robust and cost effective which makes it difficult to choose the best vector. We have therefore, undertaken a comparative analysis of a variety of available transient expression vectors. These included the vectors pJLTRBO, pPZP3425, pEAQ-HT, and pBY030-2R. In addition, we transferred the TMV expression cassette from pJLTRBO, which is a single copy vector, into the pPZP vector backbone. This vector was called pPZP5000. We compared the expression of GUS and GFP in *Nicotiana benthamiana* by Agrobacterium-mediated transformation. We found that pJLTRBO and pPZP5000 had a comparable expression level without RNAi inhibitor. The other vectors needed co-infiltration of an RNAi inhibitor expression construct to give good expression levels. The only vector which is not based on a virus genome, pPZP3425, gave also satisfactory results.

Effect of temperature on clubroot (*Plasmodiophora brassicae*) symptom initiation on Shanghai pak choy

K. Sharma (1), B. D. Gossen (2), M. MCDONALD (3)
(1) University of Kassel, Witzenhausen, GERMANY; (2) Agriculture & Agri-Food Canada, Saskatoon, SK, CANADA; (3) University of Guelph, Guelph, ON, CANADA
Phytopathology 100:S117

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important disease of Brassica crops worldwide. Studies were conducted to assess the effect of temperature on initiation of visible clubroot symptoms on Shanghai pak choy (*Brassica rapa* L. subsp. *Chinensis* (Rupr.) var. *communis* Tsen and Lee). Three-day-old seedlings were transplanted into small plastic pots (root-trainers) containing soil-less growing media, kept at 20°C for 1 wk, and inoculated by pipetting 600 µL of resting spore suspension (10^8 spores of *P. brassicae* /mL) onto the base of each seedling. After inoculation, the seedlings were transferred to growth cabinets at 10, 15, 20, 25 and 30°C (14-h photoperiod, 65% RH). Each day from 8 to 36 days after inoculation (DAI), the roots of 12 plants per treatment were collected, washed, and assessed for symptom development. No symptoms were observed at 36 DAI in plants kept at 10°C. Swelling of the tap root was visible at 28 DAI in plants at 15°C, 14 DAI at 20° and 30°C, and 10 DAI at 25°C. This result supports the results from companion studies, including field trials, that cool temperatures result in slower symptom development of clubroot in Brassica crops. Sectioning and staining to assess the impact of temperature on each stage of the pathogen's life cycle are in progress.

Genetically distinct *Cercospora* species cause grey leaf spot of maize (*Zea mays* L.) in Nigeria

K. SHARMA (1), M. Ayodele (1), R. Bandyopadhyay (1), A. E. Anu (1), A. Menkir (1), P. Lava Kumar (1)
(1) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA
Phytopathology 100:S117

Grey leaf spot (GLS) is a destructive disease of maize in many parts of Africa and the Americas. The causal agent of GLS was generally regarded as *Cercospora zeae-maydis*. However, recent studies demonstrated that two distinct species, *C. zeae-maydis* (previously Group I) in the Americas and *C. zeina*, (referred previously as *C. zeae-maydis* Group II) in Southern Africa, U.S.A. and Brazil. GLS in Nigeria was first observed in 1996 and C. zeae-maydis was considered to be the causal organism based on morphological and growth characteristics of the single-conidial cultures recovered from infected maize leaves in 2002–2004 epidemics. We assessed genetic relatedness of these isolates by nucleotide sequencing and cluster analysis of the nuclear ribosomal internal transcribed spacer region (ITS1, ITS2, and the 5.8S gene) and partial gene sequences of elongation factor 1-α, actin and beta tubulin. The nucleotide sequences of Nigerian isolates are highly homogenous and closely (96%) related to *C. apii*, *C. beticola* and *C. sorghi f. maydis* (ex. maize, Kenya) but less similar (85% to 89%) with *C. zeae-maydis* and *C. zeina*. Cluster analysis based on the four gene sequences, the Nigerian isolates formed a new phylogenetic lineage in maize infecting *Cercospora* species complex. Our results suggest that GLS in Nigeria is caused by a unique species of *Cercospora*, and *C. zeae-maydis* and *C. zeina*, were not present. A polymerase chain reaction based diagnostic assay was developed to distinguish all three species.

Genetic characterization of *Fusarium verticillioides* associated with ear rot of maize in Nigeria

K. SHARMA (1), M. Ayodele (1), P. Lava Kumar (1)
(1) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA
Phytopathology 100:S117

Fusarium verticillioides (sexual stage *Gibberella moniliformis*) is a common fungal pathogen associated with diseases such as ear and kernel rot and also responsible for mycotoxin contamination in maize (*Zea mays* L.) in Nigeria. *Fusarium* isolates from symptomatic and asymptomatic ears of maize obtained from different maize growing regions in Nigeria had similar morphological and growth characteristics. Genetic diversity was assessed by sequencing the nuclear ribosomal DNA internal transcribed spacers (ITS-1 and ITS-2), and partial gene sequences of elongation factor 1-α, actin and histone. Cluster analysis grouped these isolates into two, one group aligned with *F. verticillioides* and the other with *G. moniliformis*. However, data of histone gene was not useful to distinguish teleomorph and anamorph stages of these fungi. Nucleotide base composition between the two forms differed by 2%. Nucleotide diversity based on elongation factor, ITS and Actin is π = 0.023387, 0.008547 and 0.002769 with number of segregating sites S = 27, 26 and 11, respectively, observed based on Tajima's neutrality test confirming the differences between the two stages.

A strain differentiating macro array for Plum Pox Virus detection

D. SHERMAN (1), D. Suciu (2), R. Vigaya Satya (3), W. Schneider (1)
(1) USDA-ARS-FDWSRU, Ft. Detrick, MD, U.S.A.; (2) CombiMatrix Corporation, Mukilteo, WA, U.S.A.; (3) Biotechnology HPC Software Applications Institute, TATRC, USAMRMC, Frederick, MD, U.S.A.
Phytopathology 100:S117

A series of oligonucleotide probes were identified for strain differentiation and detection of Plum Pox Virus (PPV). Probes were designed using two different identification programs; Probe Design Suite and Tool for Oligonucleotide Fingerprint Identification (TOFI). The probes were selected to have melting temperatures (Tm) of 70–75°C, lengths of 35 – 40 bases, and GC content of 45 – 50%. The Probe Design Suite identified 10 general PPV probes and 10 *Prunus* sp. probes that met these criteria were used for further study. TOFI identified 67 strain specific probes and 13 multi-strain probes. Specificity was tested using healthy *Prunus persica* tissue and four PPV isolates: Penn4 (D), European D, M and EA. Oligonucleotide probe testing started with an open-ended SYBR green PCR array to determine probe specificity. Batch first strand-DNA was prepared using PPV specific primers that amplify across the entire genome for all the strains. Probes that were found to be specific by PCR array were then tested in a nylon macroarray format. Initial tests using the macroarray demonstrated low background signal and adequate sample labeling using a colorimetric detection method. The long term goal is to generate an optimized strain-differentiating PPV pathogen macroarray for plant diagnostic centers and/or nursery certification programs.

The virulence mechanisms of Xylella fastidiosa in xylem fluid from resistant and susceptible grapevines

X. SHI (1), Z. Liang (2), J. Bi (3), J. G. Morse (4), D. A. Cooksey (5)
(1) Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY, U.S.A.; (2) Horticulture Department, Cornell University, Ithaca, NY, U.S.A.; (3) University of California Cooperative Extension, Salinas, CA, U.S.A.; (4) Department of Entomology, University of California–Riverside, Riverside, CA, U.S.A.; (5) Plant Pathology Department, University of California-Riverside, Riverside, CA, U.S.A.
Phytopathology 100:S118

Xylella fastidiosa (Xf) is a fastidious, xylem-limited, non-flagellated, insect-transmitted, Gram-negative bacterium that causes many plant diseases, including Pierce's disease (PD). It was investigate that while grapevine V. vinifera is susceptible to the PD strain of Xf, V. champinii and V. smalliana are tolerant or resistant to the PD strain. The virulence mechanisms of Xf PD strain between resistant and susceptible grapevines were investigated by examining the in vitro effect of pure xylem fluid from resistant and susceptible grapevines on Xf multiplication, aggregation, and attachment of PD strain. The aggregations of large clumps were formed in pure xylem fluid from susceptible grapevine, whereas small clumps were observed in resistance grapevines xylem fluid. Macroarray was being applied for analysis of the differential gene expression files of Xf PD strain in differential xylem fluids of grapevines. Xylem fluid was also be analyzed to determine the chemical compounds or elements that control the virulence of Xf.

Identification and characterization of *Fusarium oxysporum*, the causal agent of koa wilt in Hawaii

A. SHIRAISHI (1), J. Uchida (1)
(1) University of Hawaii at Manoa, Honolulu, HI, U.S.A.
Phytopathology 100:S118

Koa (*Acacia koa*) is endemic to Hawaii and plays extremely important roles from economical, cultural, and ecological standpoints. However, serious wilt and dieback have been caused by a fungal pathogen, *Fusarium oxysporum*, and making the establishment of koa plantations extremely difficult. The objectives of this study are to develop a DNA-based system for rapid detection of *F. oxysporum* causing koa wilt, and to determine genetic diversity of *F. oxysporum* isolated from wilt koa. The genetic diversity within the species *F. oxysporum* includes the diversity in pathogenicity to koa. Pathogens were isolated from surface sterilized specimens collected from wilt koa trees in the Hamakua research station, the island of Hawaii. Out of 157 isolates obtained, 78 were *Fusarium* species. Pathogenicity tests were conducted with the *Fusarium* isolates by adding 10 ml of 10^5 spores/ml of each isolate to 10 koa seedlings under greenhouse conditions. After 3 months, koa mortalities varied from 0% to 70%. Also, seedlings showed various symptoms. DNA sequencing confirmed that 10 isolates, including the strongest pathogen and moderate ones, used in the test were *F. oxysporum.* This study has confirmed that pathogenicity varies within the species. In future works, AFLP analysis and VCG test will be conducted with the isolates to develop molecular markers that will allow koa growers/researchers to test if soils are contaminated with pathogenic *F. oxysporum* before planting and help establishing healthy koa plantations.

Screening of Medicago Tnt1 lines identifies genes involved in molecular interactions between *Macrophomina phaseolina* and its plant host

B. SHUAI (1), A. Reyes Gaige (1)
(1) Wichita State University, Wichita, KS, U.S.A.
Phytopathology 100:S118

Macrophomina phaseolina is a soil born necrotrophic fungus that causes charcoal rot disease in a wild range of plant hosts. Unlike most fungal pathogens, *M. phaseolina* prefers hot and dry conditions. Therefore, charcoal rot disease is most prominent in places with hot and dry summers. Although charcoal rot disease is one of the leading causes of reduced crop yield in the U.S. and around the world, there is no effective method for preventing and treating the disease. Moreover, very little is known about the molecular mechanisms involved in host-pathogen interactions. Using *Medicago truncatula* as a model, we established a genetic screen to identify genes that are involved in disease development. In our initial screen of 250 Medicago Tnt1 transposon insertion lines, we have identified 7 lines that have shown altered susceptibility to *M. phaseolina*. Comparing to the wild type plant, 6 out of the 7 lines were more resistant and 1 line was more susceptible to the fungal pathogen. Molecular techniques are applied to identify genes that are responsible for the changes. The knowledge gained from this study will become valuable for crop improvement in the future.

Disease survey of commercial soybean fields in Alabama in 2009

E. J. SIKORA (1), J. F. Murphy (1), K. S. Lawrence (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S118

A statewide survey of commercial soybeans was conducted in 2009. Observations on incidence and severity of foliar diseases, viruses, and nematodes were taken from 40 fields located in major soybean production areas in Alabama. A one acre section of each field was used for disease evaluation of foliar diseases; 50 trifoliate leaves were collected for virus testing [*Bean pod mottle virus* (BPMV), *Soybean mosaic virus* (SMV) and *Tomato spotted wilt virus* (TSWV)]; and soil samples were collected for nematode screening. Cercospora leaf blight (*Cercospora kikuchii*) was the most common foliar disease, observed in 77% of fields with disease severity in the moderate-to-high range in 45%. Downy mildew (*Peronospora manshurica*), target spot (*Corynespora cassiicola*) and soybean rust (*Phakopsora pachyrhizi*) occurred in over 40% of fields with disease severity typically at low levels. Stem canker (*Diaporthe phaseolorum*) occurred in 22% of fields with incidence between 5–35%. BPMV was detected in 65% of fields with incidence between 2–54%. SMV was detected in 48% of fields with incidence between 2–88% and TSWV was found in 12% of fields with incidence between 5–11%. Reniform (*Rotylenchus reniformis*), root-knot (*Meloidogyne* spp.) and soybean cyst (*Heterodera glycines*) were found in 30, 16 and 8% of the fields. This was the second year of a two-year study and showed that soybeans in Alabama are exposed to multiple plant pathogens with incidence and severity depending on prevailing weather conditions as well as other factors.

Histochemical detection of H_2O_2 and O_2^- in barley leaves infected with *Cochliobolus sativus*

P. Silva (1), S. RODRIGUEZ (1), C. Torres Puyo (1), F. Gamba (1), C. Pritsch (1)
(1) University Uruguay, Montevideo, URUGUAY
Phytopathology 100:S118

Spatiotemporal accumulation of H_2O_2 in barley leaves has been reported as an early response to infection by *Cochliobolus sativus*. However, these analyses have included a limited number of barley genotypes. Also, little is known whether O_2^- follows the same accumulation pattern as H_2O_2. The aim of this study is to describe *in planta* H_2O_2 and O_2^- accumulation in relation to *C. sativus* infection process. Additionally, we aim to compare the temporal and spatial pattern of H_2O_2 accumulation among NDB112 (resistant), cv. Daymán (intermediately resistant) and line CLE253 (highly susceptible). Primary leaves spray inoculated with *C. sativus* spores were sampled at 24 and 48 hai for H_2O_2 detection by pre-treatment with 3, 3-diaminobenzidine (DAB) and at 48 hai for O_2^- detection by reduction of nitrotetrazolium blue chloride (NBT). Hyphae were visualized by staining with Calcofluor. At pre-penetrated epidermal cells (cv Dayman), both H_2O_2 and O_2^- localized exclusively under appresoria. In contrast, whole cell accumulation of H_2O_2 was observed at uninfected, underlying mesophyll cells while O_2^- was restricted to chloroplasts of neighbouring cells. At later stages, whole cell staining for H_2O_2 accumulation was evident at both single epidermal cells exhibiting intracellular hyphae and infected mesophyll cells. Similar spatiotemporal distribution of H_2O_2 was observed for cv CLE253. In contrast, H_2O_2 accumulation was not detected in underlying, uninfected mesophyll cells in NDB112-inoculated tissues.

Evaluation of effectiveness of carbendazim comparatively to trifloxystrobin + tebuconazole for control of citrus postbloom fruit drop in Brazil

G. J. SILVA JUNIOR (1), M. B. Spósito (2), D. R. Marin (2), L. Amorim (1)
(1) Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Piracicaba-SP, BRAZIL; (2) Fundo de Defesa da Citricultura (Fundecitrus), Araraquara-SP, BRAZIL
Phytopathology 100:S118

Postbloom fruit drop of citrus, caused by *Colletotrichum acutatum*, is controlled by fungicide sprays during flowering. Nowadays carbendazim is most commonly used fungicide in the state of São Paulo, Brazil. In this study the effectiveness of carbendazim was assessed *in vitro* and in orchards and compared to trifloxystrobin + tebuconazole. Mycelial growth inhibition by carbendazim (1 to 1000 µg/mL) was tested *in vitro*. Field experiments were carried out in two sweet orange orchards in the state of Sao Paulo in 2009. Citrus trees received 2, 3 or 4 sprays of carbendazim (1000 g/ha) or trifloxystrobin + tebuconazole (80 + 160 g/ha) at 7-days interval, starting at the green bud stage. The percentage of symptomatic petals, number of persistent calyces and fruits per branch were determined. Carbendazim reduced the colony diameter by about 50 to 60% at all concentrations, without complete inhibition. In the field, the PFD incidence and the fruit set on carbendazim sprayed trees did not differed from disease incidence and fruit set in control trees. Four sprays of trifloxystrobin + tebuconazole mixture significantly reduced the proportion of symptomatic flowers by 94 and 71% and persistent calyces by 86 and 40%, in two orchards. Fruit set in control trees were 85 and 98% lower than fruit set in trees treated with four sprays of fungicide mixture. The data suggest that trifloxystrobin + tebuconazole can be

used to control the disease, replacing carbendazim which showed to be ineffective. Supported by FAPESP.

Spatial and temporal dynamics of postbloom fruit drop in sweet orange orchards in Sao Paulo State, Brazil

G. J. SILVA JUNIOR (1), M. B. Spósito (2), D. R. Marin (2), L. Amorim (1)
(1) Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP), Piracicaba-SP, BRAZIL; (2) Fundo de Defesa da Citricultura (Fundecitrus), Araraquara-SP, BRAZIL
Phytopathology 100:S119

Epidemics of postbloom fruit drop (PFD), caused by *Colletotrichum acutatum*, occur suddenly and can cause losses of up to 100%. The objective of this study was to analyze the spatial and temporal dynamics of PFD in young orchards, in order to understand the spread mechanisms of the disease. Symptoms on petals were assessed in 2008 and 2009 bloom seasons in two orchards of 2- and 3-yr-old Pera sweet orange with 500 trees each (20 rows with 25 trees). Population dynamics models were fit to PFD progress curves by non linear regression. The index of dispersion for binomial distribution (ID) as well as the Taylor's power law relationship between variances were calculated to determine the disease pattern. The model that best fit the data was the logistic, with initial inoculum ranging from 0.001 to 0.02 and progress rates from 0.11 to 0.45. PFD progress rates were similar to those reported for *Phytophthora infestans* in potato and considered high for a pathogen that depends on rain for dispersal. The ID was equal to 1.0 in most of the cases and the regression of the variance for the Taylor's power law had the parameters log(A)=0 and b=1 ($p < 0.05$), suggesting random distribution. There are two possibilities to justify this behavior: (i) the pathogen has additional mechanisms of dispersal, such as bees, for example, (ii) the inoculum is present uniformly in the orchards, due to pathogen survival in seedlings, soil or weeds. Both possibilities are under investigation. (Supported by FAPESP, Brazil).

***Pantoea agglomerans*, a maize seed transmitted bacterium in Mexico**

H. V. SILVA-ROJAS (1), G. Mahuku (2), P. D. Esker (3)
(1) Colegio de Postgraduados, Montecillo, MEXICO; (2) Centro Internacional de Mejoramiento de Maiz y Trigo, Texcoco, MEXICO; (3) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S119

Pantoea agglomerans has been reported to cause leaf blight and vascular wilt of maize in the Central Highland Valley of Mexico. Symptoms were consistently observed in maize from commercial and seed production fields at elevations ranging from 100 to 2,700-m. An experiment was conducted under greenhouse conditions to determine whether *P. agglomerans* was seed transmitted, and also the transmission efficiency using seed from different cultivars and with different disease incidences. Three cultivars, HS2 and Triunfo (three-way) hybrids, and Cacahuacintle, a landrace, were planted in a split plot design with three replications, and inoculated with three strains of *P. agglomerans*. A total of 1,200 seeds of each treatment were planted in trays containing sterilized soil. Initial symptoms of chlorotic streaks were observed in seedlings 15 days after emergence, and evaluations were conducted for a period of five weeks. To confirm the presence of *P. agglomerans* in chlorotic streaks, small portions of tissue were taken from the edge of the lesions, and placed on CPG medium. Sequencing of the 16S rDNA was conducted for specific identification of *P. agglomerans* isolated from chlorotic streaks. Results revealed a strong strain x cultivar interaction, and the rate of seed transmission ranged from 6 to 26% for the cultivars Triunfo and HS2, respectively. These results indicate that *P. agglomerans* is seed transmitted and at a higher rate than what has been reported for other species of *Pantoea*.

Volatile hexanal to postharvest control of brown rot of peach caused by *Monilinia fructicola* and *M. laxa*

J. SILVEIRA BAGGIO (1), S. de Afonseca Lourenço (1), L. Amorim (1)
(1) Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ/USP), Piracicaba, BRAZIL
Phytopathology 100:S119

Brown rot, caused by *Monilinia fructicola*, *M. laxa* and *M. fructigena*, is one of the most important peach diseases. In Brazil, peaches are affected by the first two species, and the second one had been reported recently. Currently, alternative methods for postharvest diseases have been studied, like the volatiles use. Tests were made to determine the effects of volatile hexanal in the development of the disease in peaches inoculated with both fungi species. Experiments were carried out with two different peach cultivars: Chiripá and O'Henry. Hexanal concentration at 50 µL/L was applied as a single dose at the moment of fruit inoculation (eradication treatment) or 24 hours after inoculation (curative treatment). Wounded and unwounded fruit were inoculated with 30 µL of conidia suspensions of each pathogen. The peaches were put inside a sealed recipient with a Petri dish containing the volatile for 24 hours at 20°C. The control treatment did not receive the volatile. Brown rot incidence and brown rot lesion diameter were assessed daily during 7 days. Overall, hexanal was more efficient in cv. O'Henry than in cv. Chiripá. Brown rot disease was reduced in average by 30% and 72% in eradication treatments for cv. Chiripá and O'Henry, respectively. In curative treatments, disease was reduced in average by 43% and 72% respectively in cv. Chiripá and O'Henry. Hexanal provides a promising alternative to chemical fungicides and can be used in postharvest handling systems. Supported by FAPESP.

***GmFAD3* genes mediate developmental and defense-related physiology in soybean**

A. SINGH (1), M. El-Habbak (1), S. Ghabrial (1), A. Kachroo (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S119

Omega-3 fatty acid desaturase (*FAD3*)-catalyzed conversion of linoleic acid (18:2) to linolenic acid (18:3) is an important step in fatty acid (FA) biosynthesis. Silencing three microsomal isoforms of *GmFAD3* using a bean pod mottle virus (BPMV)-based vector, increased 18:2 levels, and lowered 18:3 levels in soybean vegetative tissues. Greenhouse grown *GmFAD3*-silenced plants produced seeds that were significantly larger in size and greater in weight. The average number of seeds obtained from the *GmFAD3*-silenced plants did not differ significantly from control plants. Thus, silencing *GmFAD3* in soybean resulted in an ~ 60% increase in seed yield. Furthermore, seeds from *GmFAD3*-silenced plants contained similar amounts of proteins, carbohydrates, FAs and oil as those from control plants. Interestingly, the *GmFAD3*-silenced plants exhibited altered defense-related phenotypes, accumulating higher levels of the phytohormones salicylic acid (SA) and jasmonic acid (JA), and increased expression of pathogenesis-related genes. Consequently, these plants exhibited enhanced resistance to an avirulent strain of *Pseudomonas syringae* and a virulent strain of *Phytophthora sojae*. Our results show that the *GmFAD3* genes modulate seed development and phytohormone-derived defense signaling in soybean.

Epidemiology of almond leaf scorch disease in the San Joaquin Valley of California

M. SISTERSON (1), J. Chen (2), K. Daane (3), R. Groves (4), B. Higbee (5), C. Ledbetter (1)
(1) USDA ARS, Parlier, CA, U.S.A.; (2) USDA ARS PWA, Parlier, CA, U.S.A.; (3) University of California, Berkeley, CA, U.S.A.; (4) University of Wisconsin, Madison, WI, U.S.A.; (5) Paramount Farming Co., Bakersfield, CA, U.S.A.
Phytopathology 100:S119

Almond leaf scorch (ALS) disease has been present in California for more than 60 years. This disease is caused by the bacterium *Xylella fastidiosa*, which causes several other important plant diseases, including Pierce's disease of grapes. The epidemiology of ALS in the San Joaquin Valley of California was investigated to determine: 1) effects of ALS on tree yield and longevity, 2) regional incidence, and 3) disease progress curves in select orchards. Yields of ALS-affected trees were significantly lower than yields of unaffected trees. Yield loss varied with cultivar and tree death due to ALS over a 5–6 year period was rare. Almond leaf scorch disease was common in the San Joaquin Valley and at least one infected tree was found in 34 of 61 (56%) orchards containing the cultivar Sonora. Incidence in surveyed orchards was typically low (<2%). Multi-year surveys in two severely affected orchards found that incidence varied with cultivar and appeared to increase at a steady rate. For example, in one orchard incidence in the cultivar Sonora increased from 5.8% in 2003 to 8.5% in 2009. Incidence in the cultivar Nonpareil in the same orchard was lower with 1.3% of trees affected in 2003 and 2.7% of trees affected in 2009. The results indicate that ALS is present in orchards throughout the San Joaquin Valley, but that incidence and yield effects vary with cultivar.

Exploring the diversity of Phytophthora and related genera in aquatic environments in Maryland, U.S.A.

D. SKALTSAS (1)
(1) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S119

In an attempt to discover the species diversity in streams of Maryland we have conducted an intensive survey in 2009. Sites were located throughout the State including different ecosystems such as oak forests, urban parks, agricultural sites, and brackish water in Eastern Shore around Chesapeake Bay. In total 27 streams were surveyed and baited from May to August. In each site, four rhododendron leaves in a mesh bag was deployed and collected after 1–3 weeks. Due to the water temperature fluctuations, sites in lower elevation in eastern Maryland were baited up to 11 times in weekly intervals. Water-soaked or necrotic tissue samples from baited leaves were plated on selective agar (PARPNH) for *Phytophthora*, and any outgrowing colonies sub-cultures after 3–5 days. In total 1,600 isolates were identified as *Phytophthora* and 450

as *Pythium* or an unidentified Oomycete. Isolates were initially identified based on ITS sequencing. This poster presents one of the most comprehensive analysis of species assemblage of *Phytophthora* that exists in diverse aquatic environments in Maryland.

Interaction between pattern, process and scale in plant disease epidemics
P. SKELSEY (1), K. A. Garrett (1), K. A. With (1)
(1) KSU, Manhattan, KS, U.S.A.
Phytopathology 100:S120

Optimal arrangement of crops and crop varieties could make agricultural landscapes more resilient to plant pathogen invasions, but is there a 'correct' scale at which to introduce artificial heterogeneities in plant communities? For example, aggregation of host fields into clusters could serve to hamper disease spread by increasing the separation distances between host areas, but aggregation of clusters into super-clusters could lead to large crop losses if just one or two areas became infected. Here we present a spatiotemporal simulation framework to investigate the scale-dependence of pattern-process relationships in plant disease epidemics. Epidemics that result from transmission of both vectorborne and airborne infectious agents are simulated using models such as a standard SEIR model and Levy flights to describe vector movement, and a previously published model for potato late blight linked to an atmospheric dispersion model. We introduce a new class of neutral landscape model that facilitates the simultaneous study of host pattern-epidemic process relationships across a continuum of spatial scales, i.e., from plant-scale through to intercontinental-scale. In some model scenarios, landscape designs that reduced disease at one spatial scale led to increased disease at other spatial scales. These initial results indicate potential for the development of new scale-appropriate management strategies.

A metabolic fingerprinting technique for functional genomics in *Fusarium verticillioides*
J. E. SMITH (1), B. H. Bluhm (2)
(1) University of Arkansas, Garfield, AR, U.S.A.; (2) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S120

The maize pathogen *Fusarium verticillioides* infects roots, stalks, ears, and kernels. In addition, the fungus produces fumonisin mycotoxins during kernel colonization. Although the regulation of fumonisin biosynthesis is increasingly well understood, little is known about broad changes in the metabolome underlying pathogenesis. The objective of this study was to develop a metabolic fingerprinting technique to simultaneously detect and quantify structurally diverse metabolites produced by *F. verticillioides*. To create a metabolic fingerprint of the fungus, a workflow based on gas chromatography-mass spectrometry (GC-MS) was optimized to detect over 70 metabolites in the wild type strain, nearly half of which were putatively identified based on matches with characterized compounds in structural databases. Then, metabolic fingerprints were obtained from genetically defined mutants of the fungus and compared to the fingerprint of the wild type. Statistically significant differences in the production of characterized and uncharacterized metabolites were reliably detected in several mutants. Additionally, changes in the metabolome of the wild-type strain in response to environmental conditions such as pH and nitrogen availability were consistently detected. This metabolic fingerprinting technique provides a novel tool for functional genomics and reverse genetics in *F. verticillioides*, in that a wide range of "hidden" metabolic phenotypes can be identified rapidly.

Resistance breakdown in *Rz*2 containing sugar beet cultivars to *Beet necrotic yellow vein virus*
M. J. Smith (1), R. Acosta-Leal (1), C. M. RUSH (1)
(1) Texas AgriLife Research, Amarillo, TX, U.S.A.
Phytopathology 100:S120

Beet necrotic yellow vein virus (BNYVV) causes the economically important disease, Rhizomania, in sugar beets. Genetic resistance to rhizomania, conferred by the dominant *Rz*1 gene, has been overcome by resistance breaking (RB) strains of BNYVV. As a result, a second resistance gene, *Rz*2, recently was introduced into commercial cultivars. In 2009, isolated plants, from fields in southern Minnesota planted to cultivars with *Rz*2 resistance, exhibited typical rhizomania symptoms. Diseased plants had a high virus titer, as measured by real time RT-PCR, and tested positive for the presence of the *Rz*2 gene. Subsequently, alleleic discrimination based on the p25 region of BNYVV RNA 3, showed that the majority of the diseased plants contained an A_{67}, typical of wild type BNYVV, instead of a V_{67}, previously associated with Rz1 RB strains of BNYVV. This suggests that the molecular determinant(s), which allows isolates of BNYVV to overcome *Rz*2 genetic resistance, differs from that reported for *Rz*1 resistance breaking isolates.

Using weather variables to predict the probability of dollar spot development
D. L. SMITH (1), J. P. Kerns (2)
(1) Oklahoma State University, Stillwater, OK, U.S.A.; (2) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S120

Dollar spot, caused by *Sclerotinia homoeocarpa*, is the most damaging disease of cool-season turfgrasses throughout the U.S. A reliable dollar spot prediction model would be useful for timing fungicide applications for high-value turfgrasses. Logistic regression was used to develop a model to predict the probability of dollar spot development on creeping bentgrass using weather variables as inputs at sites in Oklahoma (2008 and 2009) and Wisconsin (2009). Numbers of dollar spot foci were counted daily in plots receiving no fungicide or treated with fungicide. Various on-site weather variables were recorded hourly. Weather data were transformed to 5-day moving averages. Disease severity/plot was converted to a binomial variable where 1 was average severity $\geq$ 1 spot and 0 was average severity $<$ 1 spot. Transformed weather data and the class variables season and fungicide, were used as independent variables with average disease severity (DS) as the dependent variable in model development. The best model included the class variable fungicide, and 5-day moving averages of daily relative humidity and minimum daily air temperature (Max-rescaled R-square=0.46; C=0.89). Minimum temperature thresholds to activate the model were set at 14°C based on field and controlled environment chamber studies. Independent validation exercises demonstrated that the model accurately recommended fungicide sprays when an action threshold of 30% was used in the model at both locations.

Complete genome sequence of *Pantoea vagans* biocontrol strain C9-1
T. H. Smits (1), F. Rezzonico (1), T. Kamber (1), C. A. ISHIMARU (2), J. E. Frey (1), A. Goesmann (3), V. O. Stockwell (4), B. Duffy (1)
(1) Agroscope Changins-Wädenswil ACW, Wädenswil, SWITZERLAND; (2) University of Minnesota, St. Paul, MN, U.S.A.; (3) Universität Bielefeld, Bielefeld, GERMANY; (4) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 100:S120

Pantoea vagans strain C9-1 is an effective, reliable biocontrol agent registered in the U.S.A. and Canada for fire blight control. We sequenced the complete genome of C9-1 (4.88 Mb) using 454 technology with 25x coverage yielding 1,224,924 reads and 207 contigs. Gap closure was done with Sanger sequencing and assembly was done using Lasergene software. The genome includes a 4.025 Mb chromosome (55.5% GC ratio, 3693 predicted CDS, 7 rRNA operons, 78 tRNAs), and 3 circular, non-self-transmissible plasmids (lacking tra, mob mobility genes). Plasmids pPag1 (168 kb), pPag2 (166 kb) and pPag3 (530 kb) have 162, 229 and 535 predicted CDS, respectively. Annotation identified a complete set of enterobacterial metabolic pathways and biosynthetic pathways for all amino acids and most cofactors. Four large regions containing phage-related genes, a single genomic island, ampicillin and tellurite resistance genes, and 41 genes encoding putative multidrug exporters were identified. The complete beta-carotenoid yellow-pigment biosynthetic pathway was identified on pPag3. Metabolic versatility is a distinctive feature of the C9-1 genome, particularly efficient utilization of sugars contributing to competitiveness in flower infection courts. Biosynthetic genes for multiple antibiotics (pantocin A, and dapdiamide) were characterized. Additional features of potential biocontrol/ecological fitness relevance were identified. Their potential role in biocontrol is under study.

White pine blister rust resistance in a seven year old field trial of 28 western white pine (*Pinus monticola)* families in the Coast Range of Oregon
R. A. SNIEZKO (1), J. Hill (2), R. S. Danchok (2), A. J. Kegley (2), S. Long (2), J. B. Mayo (2), A. J. Smith (3)
(1) USDA FS Dorena Genetic Resource Center, Cottage Grove, OR, U.S.A.; (2) USDA Forest Service/Dorena Genetic Resource Center, Cottage Grove, OR, U.S.A.; (3) Plum Creek Timberlands, Cottage Grove, OR, U.S.A.
Phytopathology 100:S120

Western white pine (WWP) is highly susceptible to the non-native pathogen *Cronartium ribicola*, cause of white pine blister rust. There are few reports from field trials to verify rust resistance from artificial inoculation programs of WWP. This is the first trial reported for the Coast Range of Oregon. WWP families from two resistance programs were planted in 2003. Families exhibiting several types of resistance, including a hypersensitive reaction (HR) that occurs in the needles, are represented. Infection events had occurred in several years, and both old cankers and recent stem infections were present; however, little mortality has occurred to this point. As of March 2010, 65% of trees were infected, with families ranging from 24 to 100% infected. The number of stem infections per tree ranged from 0 to 91. The susceptible control family had the highest percentage of trees infected and the most severe infections. The families with HR resistance showed relatively high infection,

indicating that a strain of the rust virulent to HR in WWP is present, the northernmost documented occurrence of *vcr2* to date. Most infections were recent and low in severity at this point. The incidence of rust suggests this site may be a moderate rust hazard and that large differences among families in rust resistance are present. This trial will continue to be monitored to assess the range and durability of blister rust resistance and the growth performance of the families.

Exploration of further sequence data for unknown regions of Ambrossia asymptomatic virus 1

N. SOKHANDAN BASHIR (1), U. Melcher (2)
(1) The University of Tabriz, Tabriz, IRAN; (2) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S121

Ambrossia asymptomatic virus 1 (AAV-1) is a novel virus discovered from Tallgrass Prairie Preserve (TPP) in northeastern Oklahoma. Sequences of four genome stretches of the virus have previously been determined from a clone library of the virus. However, there were two gaps in the genome with unknown sequences. The purpose of the present study was to complete the genome sequence by exploring sequences data for these gaps. Accordingly, a number of ten primers were designed corresponding to the known regions of the virus. Then, polymerase chain reaction (PCR) was performed by the use of different combination of the new primers or one of these primers coupled with a universal primer M13F or M13R. As a result, amplification with M13R/ R3 and F1/ R5 ended in ~600 and ~550 bp fragments, respectively. These sequences were subcloned in pCR2.1-TOPO vector (Invitrogen) and subjected to sequencing. Analysis of sequence data revealed that M13R/ R3-amplified fragment covers most of gap2 between Flex 4 and Flex 5 fragments. The fragment amplified by primers F1 and R5 covered whole of gap1 region, between Flex 2 and 3. This research has been a part of a major study to discover viruses of wild plants from TPP.

Characterization of *Phytophthora infestans* from Northern Thailand based on their mating type, metalaxyl sensitivity, and mtDNA haplotypes

J. SOPEE (1), S. Sangchote (1), P. Chiampiriyakul (2)
(1) Department of Plant Pathology, Kasetsart University, Bangkok, THAILAND; (2) Department of Plant Protection, Maejo University, ChiangMai, THAILAND
Phytopathology 100:S121

Isolates of *Phytophthora infestans*, causal agent of potato late blight, was collected in northern Thailand from 2006 to 2009. Thirty-three isolates were analyzed for mating type, metalaxyl sensitivity and mitochondrial DNA haplotypes. Metalaxyl sensitivity testing of these isolates by on the agar amended with metalaxyl at different levels of concentration. It indicated that 90 percent isolates distributed in 6 locations were intermediate sensitive to this fungicide and 10 percent located in 2 locations were sensitive. All of these isolates were A1 mating type and mitochondrial DNA haplotype IIa. This investigation on characteristics variable of P. infestans isolates obtained in Thailand which imports potato seed tubers from the major potato producing countries.

Identification and disruption of a cercosporin polyketide synthase and ABC transporter in *Cercospora coffeicola*

A. G. SOUZA (1), S. Herrero (2), L. A. Maffia (3), M. E. Daub (2)
(1) North Carolina State University / Universidade Federal de Vicosa, Raleigh, NC, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) Universidade Federal de Vicosa, Vicosa, BRAZIL
Phytopathology 100:S121

Brown eye spot of coffee, caused by *Cercospora coffeicola*, causes significant losses due to a decrease in quality and quantity of coffee produced. Many *Cercospora* species produce cercosporin, a photoactivated toxin thought to be involved in pathogenesis. The objective of this study is to determine the role of cercosporin in *C. coffeicola* pathogenesis by creating disrupted strains unable to produce the toxin. We first evaluated six Brazilian *C. coffeicola* isolates from fields representing organic and conventional production systems in the Minas Gerais state for their ability to produce cercosporin in vitro. Production varied among isolates, ranging from 3.5 - 25.3 µM/ 5 mm plug; production was undetectable in one isolate. The highest producing isolate is being used to identify homologs of a polyketide synthase (CTB1-2196aa) and ABC transporter (ATR1-1456aa) genes involved, respectively, in production and sensitivity to cercosporin in *C. nicotianae*. The *C. coffeicola CTB1* and *ATR1* genes were amplified using degenerate and standard PCR primers. We are presently sequencing these genes and analyzing them against available sequences. In addition, we have successfully transformed this isolate with a GFP construct using PEG-mediated protoplast transformation, and are currently using a *CTB1* disruption construct to generate disrupted strains. In the future, these strains will be tested for their ability to parasitize coffee plants in Brazil. Sponsor: CNPq.

Mapping the future: Metamodels for scaling potato late blight risk analysis in climate change scenarios

A. SPARKS (1), G. Forbes (2), R. Hijmans (3), K. Garrett (1)
(1) Kansas State University, Manhattan, KS, U.S.A.; (2) International Potato Center, Lima, PERU; (3) University of California, Davis, CA, U.S.A.
Phytopathology 100:S121

The general problem of how epidemiological processes scale is a topic of great interest in ecology. For example, while many disease forecasting models predict in-season disease risk using hourly or finer resolution weather data, global weather or climate change model datasets are often only available as monthly values. We created metamodels to adapt an established potato late blight risk model, Simcast, for use with daily and monthly forms of data using generalized additive models. The daily and monthly weather metamodels have R-squared values of 0.62 and 0.78 respectively. The metamodels were used to create global late blight risk maps under current and climate change scenarios for resistant and susceptible varieties. Changes in global late blight risk for areas where wild potato species are indigenous and countries where chronic malnutrition is a problem were also evaluated. While geographic regions tend to maintain their level of risk relative to other regions, some areas experience greater increases (such as Nepal) and decreases (such as Malawi) in risk. Areas of high wild potato species richness show an increased late blight risk. This metamodel framework was useful for evaluating the relative risk of late blight under climate change scenarios and can be adapted to other pathosystems.

Fungicide management strategies for the control of Fusarium head blight in southern Brazil

P. SPOLTI (1), L. Simon (1), J. dos Santos (1), N. Barros (1), D. S. Guerra (2), E. M. Del Ponte (1)
(1) UFRGS, Porto Alegre, BRAZIL; (2) BASF, Passo Fundo, BRAZIL
Phytopathology 100:S121

Effective management of Fusarium head blight (FHB) epidemics in wheat (caused by *Fusarium graminearum*) in Brazil may be achieved by using genetic and chemical control. Four non-inoculated field trials were conducted in 2009 at different locations with the aim to assess and compare the effect of two fungicides (mixture = metconazole + pyraclostrobin (BAS556) x metconazole (Caramba)); two dosages (0.5L/ha x 0.75L/ha) of the mixture; and number of applications (1 = flowering x 2 = flowering + 10 days later) on disease control and yield. A single and different wheat cultivar was used per location. FHB index varied from 5.1 to 7.3 in the two locations (with moderate resistant varieties) and from 10.1 to 31.2 in two locations (susceptible varieties). All treatments significantly reduced disease related to the unsprayed check. The use of two applications gave better results, and the use of mixture in highest dosage reduced around 92% and 76% of FHB index in susceptible and moderate resistant genotypes, respectively, and did not differ from the triazol. Fungicides had both neutral and positive effect in reducing infection by *F. graminearum* in grains. The better performance in disease control by using two applications of the mixture is possibly related to extended protection, which resulted in an average increase of 27% in test weight compared to metconazole. Mycotoxin analyses are underway and results will be presented together with other effects on yield components.

Evaluation of selective media and selective chemicals on the isolation of *Rhizoctonia* spp. from soil

T. N. SPURLOCK (1), C. S. Rothrock (2), W. S. Monfort (3)
(1) University of Arkansas, Cave Springs, AR, U.S.A.; (2) University of Arkansas, Fayetteville, AR, U.S.A.; (3) University of Arkansas, Lonoke, AR, U.S.A.
Phytopathology 100:S121

Characterizing *Rhizoctonia* spp. in field soil has been performed many times using methods such as baiting, elutriation, direct plating or direct observation. In most cases, selective media are used to suppress bacteria, antagonistic fungi, such as *Trichoderma* spp., and other fast growing fungi and oomycetes that could limit the ability to properly assess populations. Assaying populations of *Rhizoctonia* spp. by several techniques using different selective media resulted in poor suppression of *Trichderma* spp. from soils and selection of specific *Rhizoctonia* species or anastomosis groups (AGs) of *R. solani*. Thus, research was initiated to evaluate these selective media and existing or additional components in these media using isolates representing new or important anastomosis groups, species, or intraspecific groups to improve characterization of soil populations of *Rhizoctonia* spp. in a variety of cropping systems and soils in Arkansas. Preliminary testing indicated that thiophanate-methyl provided suppression of fungi similar to benomyl, but both favored isolation of *R. oryzae*. Ethanol potassium nitrate medium amended with 8 ppm prochloraz suppressed growth of *Trichoderma* spp., but not fast growing Zygomycetes. The value of existing and amended selective media using the toothpick and multiple-soil pellet methods for these soils with a history of soybean and rice production will be discussed.

Identification of potential virulence genes of *Candidatus* Liberibacter asiaticus, the pathogen associated with citrus greening (Huanglongbing)
A. SREEDHARAN (1), N. Wang (1)
(1) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S122

Citrus greening or huanglongbing (HLB) is a devastating disease of citrus, and poses a major threat to the citrus industry in the United States. *Candidatus* Liberibacter asiaticus is the strain associated with HLB in the U.S. The bacterium was introduced to Florida in 2005. Unsuccessful attempts to culture *Ca*. L. asiaticus have notably hampered efforts to understand its biology and pathogenesis mechanism despite some limited progresses in culturing. In order to identify the potential virulence genes, the genome sequence of *Ca*. L. asiaticus was screened, and about 30 candidate genes were identified. Selected genes were then tested on *Nicotiana benthamiana* for symptom expression using transient assays, and two of the candidate genes were chosen for further characterization. The leaves, petiole and roots of these infected plants were observed under the light microscope, to determine the effect of these proteins on the plants. Reverse-transcriptase PCR was conducted to confirm the expression of the genes *in planta*. The ability of the proteins to cause symptoms will be further confirmed by another transient assay, using a different vector. Real-time quantitative PCR will also be used to quantify the expression of these potential virulence genes. Identification and characterization of the various virulence genes in *Ca*. L. asiaticus will be the first step towards understanding the biology, and the basis of interaction of this pathogen with its host.

Evaluating *Thrips tabaci* and *Frankliniella fusca* (Thysanoptera: Thripridae) as vectors of Iris yellow spot virus
R. SRINIVASAN (1), H. Pappu (2), D. G. Riley (1), R. D. Gitaitis (1)
(1) University of Georgia, Tifton, GA, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S122

Thrips-transmitted *Iris yellow spot virus* (IYSV) (Family *Bunyaviridae*, Genus *Tospovirus*) affects onion production in Georgia and other parts of the United States. The presence of IYSV was first documented in Georgia in 2003. At least three major thrips species that transmit tospoviruses, the onion thrips *Thrips tabaci* (Lindeman), the tobacco thrips *Frankliniella fusca* (Hinds), and the western flower thrips *F. occidentalis* (Pergande) are prevalent in Georgia. *Thrips tabaci* is the only confirmed vector of IYSV. The transmission efficiency of nymphs and adults of *T. tabaci* was evaluated by using lisianthus, (*Eustoma grandiflorum*) as an indicator host. Results showed that both nymphs and adults transmitted IYSV at 63% and 66% respectively. The infection status of the test plants were evaluated by using IYSV-specific antiserum through a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA). To assess the vector status of *F. fusca*, nymphs and adults from IYSV-infected lisianthus were tested with antiserum specific to the non-structural (NSs) protein of IYSV through an antigen-coated plate ELISA. Both nymphs and adults tested positive at 23% and 40% respectively. These results indicate that *F. fusca* can potentially transmit IYSV. Experiments with lisianthus plants further confirmed IYSV transmission by *F. fusca*. However, the transmission rate (6%) was significantly lower than that of *T. tabaci*.

Saccharin-induced systemic acquired resistance in soybean
P. SRIVASTAVA (1), S. George (1), J. J. Marois (1), D. L. Wright (2), D. R. Walker (3)
(1) IFAS, University of Florida, Quincy, FL, U.S.A.; (2) Quincy, FL, U.S.A.; (3) USDA-ARS, Urbana, IL, U.S.A.
Phytopathology 100:S122

Systemic acquired resistance (SAR) is a widely distributed plant defense system that confers broad-spectrum disease resistance. Saccharin is known to induce a SAR response in many plant species. To evaluate the potentiating capability of saccharin in this respect, soybean (Glycine max) plants were inoculated with Phakopsora pachyrhizi urediniospores after application of saccharin, and were later rated for differences in the development of soybean rust symptoms. Plants were grown hydroponically in half strength Hoagland's solution and were challenged with pathogen at 1, 5, 10 and 15 d after treatment with 3 mM saccharin applied as either a foliar spray or a root drench at the 2nd trifoliate (V2) and early reproductive (R1) stages. Plants were destructively harvested and assessed for rust infection 2 wk after inoculation. Leaf position and mode of saccharin application were significant factors in determining the severity of rust infection. Saccharin applied as a root drench was more effective than the leaf treatment at inducing the SAR, with increased resistance observed 1 d after application and still apparent 15 d after application. In contrast, foliar treatment with saccharin did not increase systemic protection until 15 d after treatment. Application of saccharin, either to the leaf or as a root drench, had no significant effect on fresh and dry weight of plants which suggests that induction of systemic resistance to rust infection using saccharin does not affect plant growth.

Ulvan inhibits the appressorium differentiation of *Colletotrichum gloeosporioides* on apple leaves
M. J. STADNIK (1), L. Araujo (1)
(1) Universidade Federal de Santa Catarina, Florianópolis, BRAZIL
Phytopathology 100:S122

The aim of this study was to monitor the development of infection structures of *C. gloeosporioides*, the causal agent of Glomerella leaf spot (GLS), on resistant and ulvan-treated susceptible apple plants. For this, the resistant seedlings were sprayed with distillated water, while susceptible ones were treated with the algal polysaccharide ulvan (10 mg/mL) or water. All seedlings were inoculated 6 days after treatment. GLS severity was assessed daily between 4 and 10 days after inoculation, based on the visual estimation of the necrotic tissue percentage. Foliar discs from fully developed leaves were collected at 24, 48 and 72 hour intervals after inoculation and microscopically examined for determining the conidial germination and appressorium formation of fungus. Ulvan significantly reduced disease severity in seedlings. Conidial germination was not changed by neither innate nor ulvan-induced resistance. The development of fungal infection structures in resistant seedlings was similar to those observed in susceptible ones. In contrast, in ulvan-treated plants the appressorium formation was strongly inhibited and germ tube was longer. The results indicate that ulvan reduces the GLS severity by inhibiting the appressorium differentiation of *C. gloeosporioides*.

***Tomato spotted wilt virus* (Bunyaviridae, *Tospovirus*) infection alters feeding behavior of its vector *Frankliniella occidentalis* (Pergande)**
C. A. STAFFORD (1), G. P. Walker (2), D. E. Ullman (1)
(1) University of California, Davis, Davis, CA, U.S.A.; (2) University of California, Riverside, Riverside, CA, U.S.A.
Phytopathology 100:S122

Specific components of insect feeding behavior are critical to their competency in transmission of plant viruses. Although most feeding behaviors are not readily visible because they occur within plant tissues, the electrical penetration graph technique allows examination of insect feeding in real time and has been used extensively for aphids, leafhoppers and whiteflies. Very few studies have been done with thrips due to their minute size. We show that not only do male and female Western flower thrips (*Frankliniella occidentalis*) differ dramatically in their feeding behavior, as has been previously shown, but infection with *Tomato spotted wilt virus* (TSWV), a persistent/propagative virus, results in significant changes to their interactions with the plant host. Specifically, our data reveal that viruliferous males make more feeding and exploratory probes, and spend more time ingesting individual cells than non-viruliferous males over an eight hour period. Conversely no change in behavior was observed for females. Furthermore, due to the changes in male feeding behavior, viruliferous male feeding is more similar to females than to non-viruliferous males. While several studies show that infected plants alter vector feeding preference and survival, this is the first study that we are aware of to report a change in the feeding behavior of a plant virus vector due to viral infection of the vector. The implications of these finding to transmission of TSWV will be discussed.

A unique microbe-microbe and host-specific rhizosphere interaction that is detrimental to plant health
M. STANGHELLINI (1), I. Misaghi (1)
(1) University of California, Riverside, CA, U.S.A.
Phytopathology 100:S122

Monosporascus cannonballus, a host-specific root-infecting ascomycete, is the causal agent of a destructive disease of melons known as vine-decline. Ascospores, which function as the sole survival propagules and primary inoculum for this soilborne fungus, germinate only in the rhizosphere of melons growing in field soil. However, no ascospore germination occurs in the rhizosphere of melons if the field soil is heated to temperatures greater that 50°C prior to infestation with ascospores. This observation indicates (i) that melon root exudates alone are not the stimulant and (ii) suggests that germination is mediated by one or more heat-sensitive members of the soil microflora. Although bacteria or actinomycetes were heretofore suspected as the germination-inducing microbe(s), our recent data demonstrate that the culprit is an obligate, holocarpic, root-infecting zoosporic fungus known as Olpidium bornovanus. Ascospore germination in sterile field soil occurred only in the rhizosphere of melon roots that were colonized by a host-specific melon strain of O. bornovanus.

Characterization of *Sclerotinia sclerotiorum* in common bean white mold resistance screening locations across the U.S.A.
J. STEADMAN (1), S. McCoy (1), B. Higgins (1), L. K. Otto-Hanson (2)
(1) University of Nebraska, Lincoln, NE, U.S.A.; (2) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 100:S122

There are currently no sources of complete resistance to *Sclerotinia sclerotiorum* in common bean and other hosts. Repeatability of resistance expression in bean lines with putative sources of white mold resistance is a constraint in multi-site screening nurseries. Pathogen variability in these nurseries is unknown. To assess pathogen variation, isolates were collected from white mold field screening nurseries in eight states and 2 countries over 4 years and analyzed using mycelial compatibility tests for genotype and straw test for measuring aggressiveness. From 146 screening nursery isolates and 9 greenhouse screening isolates, 64 mycelial compatibility groupings (MCGs) were identified. An additional 84 isolates collected from bean grower fields were tested against isolates in the previous 64 MCGs and an additional 22 MCGs were found, for a total of 86 MCGs from 240 isolates. Isolates within an MCG did not differ significantly in aggressiveness, however, isolates in different MCGs were significantly different in aggressiveness. Four microsatellite primers were used to characterize the 240 isolates and they formed 67 haplotypes defined by the number of alleles at each locus. The molecular variance (AMOVA) results confirmed the earlier findings from the MCGs that there is more variation within populations (76%) than between populations (24%). Of the 86 MCGs, 68 have one allelic haplotype and of the remaining 17, 10 differed by one repeat at a locus that was found to have 20 different alleles.

Incorporation of edible brassicacious greens did not control nematode populations

K. STEDDOM (1), K. Ong (2), J. Starr (3)
(1) Texas AgriLife Extension Service, Overton, TX, U.S.A.; (2) Texas AgriLife Extension Service, College Station, TX, U.S.A.; (3) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S123

Nematodes cause significant damage on a wide range of vegetable crops including many grown in home gardens. Many studies have shown that incorporation of brassicacious green manures can reduce populations of nematodes, fungi, and other soil-borne pests. The purpose of this study was to determine if edible greens commonly grown by home gardeners could also function as effective green manure crops. Seven different edible greens crops were planted in the fall of 2007 and incorporated in the spring of 2008 and pumpkins were seeded four weeks later. Plots with Dwarf Blue Kale had higher total populations of root knot, ring, and stunt nematodes prior to incorporation of the crop. This was not correlated with populations from the previous fall suggesting an increase on either the kale or weeds present due to poor stand establishment. At the end of the trial, populations of root knot, ring, and stunt nematodes were not significantly different between any treatments. The total number of small pumpkins was greater with Vates collards or dwarf blue kale than dwarf Siberian improved kale, purple top turnip, or florida broadleaf mustard. The numbers of medium and large pumpkins or the total number of pumpkins per plot were not significantly different between treatments. An outbreak of southern blight, another soil-born pest, was widespread and not effected by treatment. Overall, incorporation of edible greens were not effective at managing populations of nematodes or *S. rolfsii*.

Investigations on population and hosts of *Bean pod mottle virus* in Mississippi

R. C. STEPHENSON (1), S. Sabanadzovic (1)
(1) Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S123

Bean pod mottle virus (BPMV) is a viral pathogen found in virtually all soybean fields in Mississippi during recent surveys. A study was undertaken to better understand the local population of this virus and to investigate sources of primary inoculum. Investigation of the local BPMV population was carried out by sequencing and comparison of four genomic regions of numerous isolates collected from research and production fields throughout Mississippi. All studied isolates showed great molecular uniformity with the exception of two, which appeared distinct in the RNA-dependent RNA polymerase region. Sequencing of the complete RNA-1 segments for these two isolates is currently on-going. In a study on sources of early infections, several plant species were identified as new hosts with possible roles in the epidemiology of this virus in the Southeastern United States.

A modified method to screen for partial resistance to *Phytophthora sojae* in soybean

S. M. STEWART (1), A. E. Robertson (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S123

Phytophthora sojae, an economically important pathogen of soybean, has been managed mainly through the use varieties with single gene (*Rps*) resistance. The pathogen, however, has the ability to rapidly adapt and overcome this type of resistance, resulting in populations that are able to infect soybean plants with the deployed Rps genes. Partial resistance (PR) is effective against all physiological races of the pathogen. Few cultivars with high levels of PR are currently available because feasible methods that enable effective incorporation of PR into desired germplasm are lacking. The layer test is presently used to screen for PR in breeding programs. In this method, soybean roots are forced to grow through a *P. sojae*-inoculated agar layer, and assessment of PR is based on root rot evaluations that require experience and can be subjective. The layer test was modified by replacing the agar layer with *P. sojae*-inoculated rice. Dry root weight was used to screen for PR. Six varieties of soybean with known levels of PR were evaluated using the layer test and modified method. No differences between methods were observed for PR, and PR ratings correlated well with corrected dry root weight ($r = -0.965$, $P < 0.001$). The modified method has the advantage that handling agar plates is not required, no training is needed for evaluations and more than one pathotype can be easily used to screen for PR by mixing rice inoculum of different isolates.

Using microsatellite markers to assess diversity of *Phytophthora sojae* in Iowa

S. M. STEWART (1), A. E. Dorrance (2), A. E. Robertson (1)
(1) Iowa State University, Ames, IA, U.S.A.; (2) OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S123

Phytophthora root rot (PRR) caused by *Phytophthora sojae* can infect soybeans at all growth stages, causing pre- and post-emergence damping-off, root and stem rot. The most effective way to manage PRR is through the use of resistant cultivars however, the pathogen continues to diversify making resistant genes present in cultivars ineffective. Deployment of durable R-gene mediated resistance depends on understanding the factors that contribute to changes in the pathogen population diversity. Twenty four single sequence repeat (SSR) loci were used to analyze the genetic diversity of sixty seven mono-zoosporic isolates of *P. sojae*, collected from diseased plants and soil throughout Iowa. Fifty percent of the loci assessed were polymorphic, and the maximum number of alleles per locus was 2, with an average of 1.5. Isolates were predominantly homozygous. The highest observed heterozygosity for an individual locus was 0.045 with an average 0.005. Selfing was estimated in $s = 0.985$, which agrees with previous reports of low levels of outcrossing due to the homothallic nature of the pathogen. The diversity of the population of *P. sojae* in Iowa will be compared to the diversity of *P. sojae* populations from Ohio, Missouri and South Dakota.

Cultivar selection for sugar beet root rot resistance

C. A. STRAUSBAUGH (1), I. A. Eujayl (1), P. Foote (2)
(1) USDA ARS NWISRL, Kimberly, ID, U.S.A.; (2) Amalgamated Sugar Company, Paul, ID, U.S.A.
Phytopathology 100:S123

Fungal and bacterial root rots in sugar beet caused by *Rhizoctonia solani* (Rs) and *Leuconostoc mesenteroides* subsp. *dextranicum* (Lm) can lead to root yield losses greater than 50%. To reduce the impact of these root rots on sucrose loss in the field, storage, and factories, studies were conducted to establish a faster and more accurate screening method. In 2009, 22 commercial cultivars were grown in a commercial field and mechanically harvested, and then inoculated. In each root a cork borer hole at the widest portion of the root was inoculated with Rs and another with Lm, while a third hole was inoculated with both (RsLm). The roots were then incubated in the greenhouse for 3 weeks out of direct sunlight, cross sectioned, and evaluated for rot. The study was repeated with roots that had been stored for 60 days. All roots suffered some rot with the Rs or the RsLm inoculations and the most susceptible cultivar had 3.9 and 2.8 times more rot than the most resistant cultivar, respectively. Only 15% of the roots developed rot with the Lm inoculation. Similar rot results for all three inoculations were obtained with stored roots. With the RsLm inoculation, cultivar ranking at harvest and after storage were correlated ($r = 0.6608$, $P = 0.0008$). The RsLm inoculation may prove to be a faster and more precise method to screen for bacterial rot resistance but screening for fungal rot resistance will likely need to be done using other methods.

Synergistic effects between Regalia® and other fungicides in controlling cucumber powdery mildew and lettuce downy mildew

H. SU (1)
(1) Marrone Bio Innovations, Inc., Davis, CA, U.S.A.
Phytopathology 100:S123

Regalia® is a biofungicide formulated from extract of giant knotweed (*Reynoutria sachalinensis*) and effectively controls common fungal and bacterial diseases. Combination (tank mix) of Regalia® and other commercial fungicides can be an effective and efficient measure in increasing pathogen control efficacy and managing fungicide resistance in pathogens. Greenhouse

and growth chamber experiments were conducted to evaluate the efficacy of Regalia® in combination with commonly used chemical and biological fungicides in controlling cucumber powdery mildew (*Sphaerotheca fuliginea*) and lettuce downy mildew (*Bremia lactucae*). The results show a statistically significant synergistic effect with Regalia® in a tank mix with azoxystrobin (Quadris®), myclobutanil (Rally® 40W), quinoxyfen (Quintec®), or triflumizole (Procure®) in controlling powdery mildew on cucumber in repeated tests. Only an additive effect with no synergy was found with tank mixes of Regalia® with *Bacillus subtilis* (Serenade®), or *Bacillus pumilus* (Sonata®), cyprodinil (Vangard®), or kresoxim-methyl (Sovran®) in non-repeated tests. Synergistic effect was also found when Regalia® was applied in combination with acibenzolar-S-methyl (Actigard®) to control lettuce downy mildew. The results clearly show that Regalia® can be effectively applied with other fungicides either in a tank mix or a rotation to increase efficacy and/or reduce the risk of fungicide resistance.

Regulation of genes involved in the interaction of tomato, *Trichoderma hamatum* 382 and *Xanthomonas euvesicatoria*

N. Subedi (1), F. BAYSAL-GUREL (1), H. Hoitink (1), M. Ivey (1), S. A. Miller (1)
(1) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S124

Trichoderma hamatum 382 (T382), a bio-control fungus, suppresses diseases in tomato by induction of systemic resistance. T382 applied as an amendment of planting mix colonized tomato roots and significantly reduced bacterial leaf spot (*Xanthomonas euvesicatoria* 110C) development without colonizing above-ground plant parts. The expression of certain tomato genes potentially involved in the interaction of tomato plants with T382 and *X. euvesicatoria* 110C was investigated using real time quantitative PCR. The genes for extensin and expansin, both cell wall proteins, and osmotin, a member of the PR-5 protein family, were not consistently activated in tomato leaves by T382 before *X. euvesicatoria* inoculation. After *X. euvesicatoria* inoculation, extensin was up-regulated in plants colonized by T382. Expansin was initially down-regulated in *X. euvesicatoria*-inoculated plants, regardless of T382 treatment; however, expression of the gene remained low over time only in *X. euvesicatoria* inoculated, T382-colonized plants. This suggests that disease suppression in tomato by T382 may involve priming plants to down-regulate expansin gene expression upon pathogen attack. Osmotin was up-regulated only in *X. euvesicatoria*-inoculated plants. T382 had no effect on expression of this PR protein by tomato plants.

Development of a biological sensor for powdery mildew (Erysiphales) infections via monitoring of the proboscis extension reflex in honeybees

A. M. SUTHERLAND (1), R. M. Wingo (2), K. J. McCabe (3), W. D. Gubler (1)
(1) Department of Plant Pathology, University of California, Davis, Davis, CA, U.S.A.; (2) Chemical Diagnostics and Engineering, Los Alamos National Laboratory, Los Alamos, NM, U.S.A.; (3) Bioscience Dision B, Los Alamos National Laboratory, Los Alamos, NM, U.S.A.
Phytopathology 100:S124

Organisms with superior sensory abilities can be developed into biological sensors, able to detect and respond to stimuli of human interest. Such sensors could be employed to provide early detection of important agricultural plant pathogens, reducing fungicide application volume and frequency, and therefore alleviating the economic and environmental costs involved therein. Through classical conditioning, domestic honeybees, *Apis mellifera*, were trained to associate olfactory cues from grape, *Vitis vinifera* "Carignane", leaves infected with powdery mildew (PM), *Erysiphe necator*, with a sucrose reward, as evident through the proboscis extension reflex (PER), an observable and unambiguous extension of the insect's proboscis. This conditioned response, however, was also elicited when bees were later presented with uninfected grape leaves. In order to reduce the incidence of such false positive responses, uninfected grape leaf material was added to the conditioning process as a continuous olfactory stimulus, serving as a constant background for the target stimulus: PM-infected leaf material. This new method has resulted in negligible levels of false positive responses while retaining acceptable levels of true positive responses. This work represents an important preliminary investigation into the feasibility of using PER observation as a biological sensor and indicator for early-season PM infections in commercial vineyards.

A comparison of soil and corn kernel *Aspergillus flavus* populations: Evidence for niche specialization

R. R. SWEANY (1), K. E. Damann (1)
(1) Louisiana State University Ag Center, Baton Rouge, LA, U.S.A.
Phytopathology 100:S124

Aspergillus flavus is a fungal, opportunistic, soil-borne pathogen of corn which may produce carcinogenic, acutely-toxic aflatoxins. The purpose of this study was to determine if there are two ecotypes of *A. flavus*: facultative parasites and saprophytes. Mature corn ears and soil samples were collected from eleven Louisiana fields in August, 2007. *A. flavus* was isolated from kernels (612 isolates) and soil (255 isolates). Sixteen vegetative compatibility groups (VCGs) were identified from soil isolates and six of these VCGs were found in corn isolates. Eighty-eight percent of corn isolates belonged to 2 VCGs (found in all fields) whereas only 5% of soil isolates were in the same 2 VCGs (found in 3 fields). Haplotypes were generated for a random subsample of 99 corn and 91 soil isolates from polymorphisms at 8 simple sequence repeat (SSR) loci. SSR fingerprints revealed 102 different haplotypes with 26 from corn isolates and 78 from soil isolates. One haplotype was shared between corn and soil isolates. Both VCG assemblages and SSR fingerprints differed significantly between the soil and the corn kernel populations. Differences between the populations indicate not all soil isolates are effective corn pathogens whereas some isolates have become effective at occupying the corn niche. Understanding the pathogenicity of *A. flavus* is important for developing atoxigenic biological control against toxigenic *A. flavus* and for resistance screening.

Forward and reverse genetic approaches for functional analyses of *Clavibacter michiganensis* subsp. *sepedonicus*

R. L. SYVERSON (1), C. A. Ishimaru (1)
(1) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 100:S124

The Gram-positive plant pathogen *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) causes bacterial ring rot of potato. The complete genome sequence of *Cms* (ATCC33113) became available in 2008, making broad-scale identification of genes affecting pathogenesis possible. To fully capitalize on the genome sequence, forward and reverse genetic approaches for functional analysis of *Cms* were developed. In each case, the sequenced strain of *Cms* was transformed via electroporation under optimized conditions. For site-directed mutagenesis, full-length *chp*7, a known pathogenicity gene in *Cms*, was cloned into pGEM®-T Easy. A chloramphenicol resistance marker originating from *Corynebacterium striatum* was inserted at a *Sma*I restriction site in *chp*7. The resulting construct was used to transform *Cms* by homologous recombination. Mutations were confirmed by PCR, sequence analysis, and plant assays to validate the utility of the approach. This same strategy was applied to other annotated virulence genes. For reverse genetics, *Cms* was transformed via transposon mutagenesis using Ez-Tn5, which inserts randomly into Gram-negative and Gram-positive bacteria. In contrast, *Tn*1409C, used previously for mutagenesis of *Cms*, inserts non-randomly into low GC regions. About 5000 Ez-Tn5 mutants of *Cms* were generated. Further library characterization will be presented. Site-directed and transposon mutagenesis can be adapted for future high-throughput functional studies.

Detection and distribution of Longidoridae and Trichodoridae nematodes from Golestan National park of Iran

A. TALEZARI (1), F. Khozeini (2), S. Barouti (3), H. Zamanizadeh (1)
(1) Dept. of Plant Pathology, Science and Research Branch, Islamic Azad University, Tehran, IRAN; (2) Iran Plant Protection Organization, Tehran, IRAN; (3) Laboratory Complex, Science and Research Branch, Islamic Azad University, Tehran, IRAN
Phytopathology 100:S124

Nematodes belonging to the families Longidoridae and Trichodoridae are always able to transmit plant viruses and for that they are of most important important plant parasitic nematodes. During the years 2008 to 2010, a total number of 50 soil and root samples were collected from Golestan National park located in North of Iran. The nematodes were extracted from roots by Coolen and De' Herde, 1972 and from soil by Jenkins, 1964 methodes. They were fixed and transfered to glycerin. The permanent slides were prepared from specimens, morphological and morphometric characters were studied by light microscope. In this study 6 species belonging to 3 genera of Longidoridae and Trichodoridae families were identified which reported for the first time from Golestan National park. The identified nematodes are as follow: Longidorus iranicus collected from Raspberry shrub and Nettle and Walnut tree; Xiphinema americanum from Oak tree ; Xiphinema diversicaudatum and X. index from Fig trees; Xiphinema pachtaicum from Common alder, Fig, Persimmon, Walnut, and Wild plum trees and Trichodorus primitivus from Maple, Nettle, Oak, Persimmon trees and Raspberry shrub. Results showed that three species Longidorus iranicus, Xiphinema pachtaicum and Trichodorus primitivus are dominat species with high population density through visited regions. According to our knowledge this the first report of these nematodes from Golestan National park of Iran with 92000 hectare wide.

Description of two genotypes of Phytomonas associated to oil palm diseases in Peru: Marchites Sorpresiva and a new disease manifestation-Marchites Lenta

M. J. TALLEDO ALBUJAR (1), S. S. Morales Ruiz (2), E. Trinidad Chipana (3), J. Arevalo Zelada (2), A. Trelles Di Lucca (3), Y. Montoya Piedra (1)
(1) Bio Links S.A., Lima, PERU; (2) Institute of Tropical Medicine "Alexander von Humboldt" Universidad Peruana Cayetano Heredia, Lima, PERU; (3) Palmas del Espino SA, Uchiza, PERU
Phytopathology 100:S125

Demand of oil palm products is worldwide increasing. In Peru the oil palm plantations are expanding, however, palm diseases represent a risk to plantations productivity. Among them, Marchites Sorpresiva (MS) was detected in Palmas del Espino plantation since 1983. Like in other Latin-American regions, Phytomonas were associated to MS. Furthermore, a second clinical manifestation appeared in 2002, which was also a vascular disease. Nevertheless, the former was characterized by a very fast process (palms died in 2 to 3 weeks) compared with the second one (palms died between 16 and 32 weeks). This fact leaded to name the latter Marchites Lenta (slow progression). Interestingly, palms affected by M. Lenta presented scarce number of Phytomonas microorganisms compared with MS. To determine if these two different disease manifestations are due to Phytomonas or correspond to two different Phytomonas, polymorphic DNA sequences were analyzed. DNA was extracted from root saps containing Phytomonas from MS (n = 22) and M. Lenta (n = 131) diseases. Specific PCR analysis of kinetoplast DNA (1000 bp and 800 bp for MS and M. Lenta oil palms, respectively) and miniexon PCR sequencing (≈100 bp for both diseases, 1 transition and 1 deletion nucleotide changes was observed in MS) demonstrated that differences were directly correlated to each disease manifestation. These findings support the hypothesis that we would be dealing with two different diseases, associated to two discrete Phytomonas groups.

High-Fidelity PCR as a sensitive molecular diagnostic tool to detect *Phytophthora nicotianae* on spathiphyllum

T. L. Tarnowski (1), A. J. PALMATEER (1)
(1) University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S125

To compare the sensitivity of High-Fidelity (Hi-Fi) and standard PCR in detecting *Phytophthora nicotianae* in symptomatic spathiphyllum (*Spathiphyllum wallisii*) plants, four DNA extraction methods were tested in conjunction with a standard and two Hi-Fi PCR protocols. The DNA extraction methods were: 1) Extract-N-Amp Plant Kit (Sigma-Aldrich), 2) DNeasy Plant Mini Kit (Qiagen), 3) CTAB buffer, and 4) lithium chloride Shorty buffer. Symptomatic leaf, petiole and root tissue from four plants were submitted to each extraction method. DNA samples were then used for each PCR protocol using *P. nicotianae* -specific primers: 1) Standard PCR, 2) Hi-Fi PCR using LongAmp enzyme, and 3) Hi-Fi PCR Taq+Accuzyme. DNA quantification using spectrophotometry indicated Extract-N-Amp and Shorty methods yielded the highest DNA amounts with lower purity. Both Hi-Fi PCR protocols were more sensitive than standard PCR. The Accuzyme protocol detected the pathogen in all samples using the DNeasy and Extract-n-Amp methods, whereas the standard protocol detected the pathogen only in leaf samples using the DNeasy kit. This study demonstrates that Hi-Fi PCR provides a highly sensitive tool for molecular diagnostics *in planta*, and that the DNA extraction method influences PCR sensitivity.

A new Bipolaris leaf spot of cordyline and disease response on five cordyline varieties

T. L. Tarnowski (1), A. J. PALMATEER (1)
(1) University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S125

A new leaf spot disease of cordyline (*Cordyline* spp.) was submitted to the Extension Plant Diagnostic Clinic in Homestead, FL. Lesions began as small pinpoint chlorotic flecks and enlarged longitudinally up to 1 cm. Lesions became necrotic with red margins and areas of dark sporulation in margin centers. A *Bipolaris* sp. was consistently isolated from lesions and confirmed as the causal agent. The species produces velvety and dark, blackish brown colonies. Conidiophores were pale golden brown, straight to flexuous, 130-285 µm × 5-9 µm. Conidia were pale brown pale to medium golden brown, smooth and clavate with a protuberant hilum, 43-86 × 11-18 m, 3-11 distoseptate. The susceptibility of five cordyline varieties, *Cordyline fruticosa* var. 'Chocolate Queen' and 'Auntie Lou', and *Cordyline australis* var. 'Dark Star', 'Redstar' and 'Sundance', was determined. Each variety was inoculated with a conidial suspension in a randomized complete block design and incubated at 27°C for 7 days. The number of lesions per leaf and total per plant were recorded. An analysis of variance indicated a significant variety effect ($P < 0.05$) and 'Chocolate Queen' had greater number of lesions than the other varieties (41 lesions compared to 10–16 lesions per plant). This is the first study that attempts to characterize the etiology and disease response of a new Bipolaris leaf spot affecting cordyline.

GFP is efficiently expressed by Wheat streak mosaic virus using a range of Tritimovirus NIa cleavage sites and forms dense aggregates in cereal hosts

S. TATINENI (1), R. French (1)
(1) USDA, ARS, University of Nebraska, Lincoln, NE, U.S.A.
Phytopathology 100:S125

Wheat streak mosaic virus (WSMV)-based transient expression vector was developed to express GFP as a marker protein. The GFP cistron was engineered between the P1 and HC-Pro cistrons in an infectious cDNA clone of WSMV. The cleavage sites, P3/6KI, 6KI/CI, NIa/NIb, or NIb/CP, from WSMV were fused to the C-terminus of GFP such that free GFP will be released after proteolytic processing of viral polyprotein. WSMV-GFP constructs infected wheat similar to that of the wild-type virus and expressed GFP mostly as aggregated structures even though proteolytically processed free GFP was detected by immuno-blots. GFP was similarly expressed as aggregates with heterologous cleavage sites from Brome streak mosaic virus or by mutating the -1 amino acid (aa) position of WSMV cleavage sites to either alanine or arginine. Binary vectors with GFP cistron containing aa that would result from cleavage sites (GFP-CS) or co-infiltration of GFP-CS with WSMV NIa cistron failed to form such aggregates in Agrobacterium-infiltrated Nicotiana benthamiana leaves, suggesting that neither aa that result from cleavage nor possible interactions between the NIa-pro and GFP-CS are involved in the formation of aggregated GFP. However, GFP was expressed mostly as free protein from WSMV-GFP with Foot-and-mouth disease virus 2A peptide (33 aa) at the C-terminus of GFP cistron. WSMV-GFP vectors were relatively stable in wheat plants and expressed GFP beyond five serial passages at 14-day intervals.

Infection of rice by *Ustilaginoidea virens*

D. TE BEEST (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S125

False smut, caused by *Ustilaginoidea virens*, has emerged as an important disease of rice in Arkansas since it was found in a single field in 1998. Little is known about the disease cycle of false smut. Experiments were conducted in the laboratory, field and greenhouse to examine infection of rice by *U. virens.* Our histological results show that rice is infected by spores germinating on root surfaces. Amplification of fungal rDNA isolated from rice tissue samples shows that infection of roots leads to the asymptomatic colonization of the entire plant including panicles and seeds. Germinating rice seeds in pasteurized soils infested with spores in concentrations ranging from 0 to 250,000 spores/gm resulted in seedling infections ranging from 0% to 100% infection. Field and greenhouse experiments showed that selected fungicides protected seedlings from infection and subsequent colonization of plants. Taken together, these findings clarify the disease cycle, disease development and epidemiology of false smut in Arkansas and may suggest new approaches to the control of *U. virens* on rice world-wide.

Baseline sensitivity of *Ascochyta rabiei* to penthiopryad, a new SDHI fungicide

N. H. Thaher (1), B. D. GOSSEN (2), M. McDonald (1)
(1) University of Guelph, Guelph, ON, CANADA; (2) Agriculture & Agri-Food Canada, Saskatoon, SK, CANADA
Phytopathology 100:S125

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labr., is a destructive disease of chickpea (*Cicer arietinum* L.). Repeated application of fungicide is required almost every year for blight management, and insensitivity to strobilurin fungicides as become widespread in North America since 2006. The sensitivity of *A. rabiei* to penthiopryad, an efficacious new succinate dehydrogenase inhibitor (SDHI) fungicide not yet in commercial use, was assessed using 50 isolates collected in 2008 in Saskatchewan, Canada. The effective concentration to inhibit mycelium growth by 50% (EC50) was estimated for each isolate using a radial growth assay on PDA amended with technical grade penthiopryad at 0.01, 0.1, and 1 µg/mL with three replicates per treatment. EC50 values ranged from 0.002 to 0.30 µg/mL with a mean of 0.10 µg/mL. A discriminatory dose of 0.3 µg/mL was selected for assessment of 32 additional isolates collected in 2008 and 47 isolates collected in 2009 (never exposed to penthiopryad); 12 of the 79 isolates exhibited < 50% growth inhibition. This study will provide the basis for monitoring sensitivity in *A. rabiei* populations to this new fungicide. Also, cross-resistance between penthiopyrad and boscalid (a SDHI used for blight management on chickpea) is being assessed.

Sensitivity of *Didymella bryoniae* to DMI and carboxamide fungicides
A. THOMAS (1), K. L. Stevenson (1), D. B. Langston (1)
(1) University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S126

Didymella bryoniae, the causal agent of gummy stem blight (GSB) of watermelon, has a history of developing resistance to fungicides, most recently the carboxamide fungicide boscalid. To facilitate fungicide resistance monitoring, baseline sensitivity distributions were established for DMI fungicides tebuconazole and difenoconazole and the carboxamide fungicide penthiopyrad that were recently introduced or are being evaluated for GSB control. In all, 77 isolates with no prior exposure to carboxamides or DMIs were tested using a mycelial growth assay to determine the effective concentration at which mycelial growth was inhibited by 50% (EC_{50}). EC_{50} values for boscalid, penthiopyrad, tebuconazole and difenoconazole ranged from 0.007 to 0.127, 0.009 to 0.189, 0.073 to 0.388 and 0.012 to 0.135 µg/ml with median values of 0.037, 0.030, 0.135 and 0.046 µg/ml. There was a significant positive correlation between the sensitivity to penthiopyrad and boscalid ($P < 0.0001$, $r = 0.63$), indicating a significant potential for cross-resistance between these compounds. In 2009, 104 isolates collected from fungicide-treated watermelon fields were tested for resistance to boscalid and penthiopyrad using a discriminatory concentration of 3.0 µg/ml. Of the isolates tested, 86 were resistant and 12 were sensitive to both fungicides. Because of the significant potential for cross-resistance, growers will be advised not to use boscalid and penthiopyrad in the same fungicide spray program.

IR-4 Project fungicide registration update
D. C. THOMPSON (1), J. Corley (1), W. Barney (1), D. Carpenter (1)
(1) Rutgers University, Princeton, NJ, U.S.A.
Phytopathology 100:S126

In 2009, the IR-4 Project obtained new uses of 29 chemicals on many specialty crops with a total of 917 new chemical uses being registered. New fungicide registrations on food crops included uses of cyazofamid, dimethomorph, fenamidone, famoxadone, propiconazole, tebuconazole, and triflumizole. Residue studies of kasugamycin on pear, apple, tomato, pepper, and walnut were completed and provided to the registrant for submission to EPA. Downy mildew of basil was identified as a problem in 2008 and residues studies for cyazofamid and mandipropamid initiated in 2009 and fluopicolide in 2010. A mandipropamid residue study was initiated for control of *Phytophthora capsici* in snap beans. IR-4 is exploring use of quarternary ammonium products directly on crops vs present use on non-porous surfaces only. The chlorothalonil risk cup was expanded and residue studies on citrus, guava and lychee were initiated in 2009 followed by almonds, mustard greens and radish in 2010. IR-4 initiated residue studies with metrafenone, a new powdery mildew fungicide from BASF. IR-4 petitioned EPA for registrations on new berry and onion subgroups adding many new minor crops. Fruiting vegetable subgroups will be established in 2010 and will include okra. Citrus, pome and stone fruit crop groups will also be expanded in 2010. An international residue program with mandipropamid and difenoconazole on tomato is underway to help determine if data from around the world can be used to establish national residue tolerances.

Mustard cover crop for management of Phytophthora blight (*Phytophthora capsici*) in cucurbit fields
S. B. THRU PPOYIL (1), M. Babadoost (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S126

This study was conducted to determine the effectiveness of two mustard species, *Brassica alba*, 'Tilney' (T), and *Brassica juncea*, 'Florida Broadleaf' (FB), as short-cycle cover crops, for managing Phytophthora blight (*Phytophthora capsici*) of cucurbits. Experiments were conducted during 2008–2009 in a *P. capsici*-infested field with a history of Phytophthora blight. Mustards were grown in the field for 45 days and were incorporated into the top 10-cm layer of the soil. The seeds of cucumber ('Eureka'), jack-o-lantern pumpkin ('Magic Lantern'), and processing pumpkin ('Dickinson') were sown after the incorporation of mustard into the soil. Average density of *P. capsici* oospores per gram of dry soil was 2.67 prior to incorporation of mustard plants. The density of oospores was 1, 1.66, 2.33, and 0 in control, T, FB, and T+FB plots, respectively, 14 days after the incorporation of mustard plants into the soil. There was no vine infection in cucumber plots. Incidence of vine infection on jack-o-lantern pumpkin plots was 52.92, 43.13, 32.70, and 51.04%; and on processing pumpkin the incidence was 45.00, 52.71, 48.33, and 47.29% in control, T, FB, and T+FB plots, respectively. Incidence of Phytophthora fruit rot on cucumber was 26.91, 20.83, 12.93 and 13.44%; on jack-o-lantern pumpkin, it was 24.43, 31.63, 22.75, and 28.80%; and on processing pumpkin, the incidence was 26.20, 18.80, 22.69, and 45.37% in the control, T, FB, and T+FB plots, respectively.

The effect of new aphid vectors on the evolution of Soybean dwarf virus
B. TIAN (1), W. L. Schneider (2), F. E. Gildow (1)
(1) Department of Plant Pathology, Pennsylvania State University, State College, PA, U.S.A.; (2) Agricultural Research Service, United States Department of Agriculture, Fort Detrick, MD, U.S.A.
Phytopathology 100:S126

Soybean Dwarf Virus (SbDV) commonly occurs in clover in North America; and prior to 2000 was not known to cause disease in soybean, presumably due to the lack of aphid vector species capable of colonizing soybean in N. America. Due to the introduction of the soybean aphid, *Aphis glycines*, into N. America, there is a critical need for evaluating the risk of aphid transmission and SbDV outbreaks in U.S. soybean fields. To determine the probability of a SbDV clover strain being selected for both efficient *A. glycines* transmission and adaptation to soybean, we passaged SbDV on soybeans using *A. glycines* and *Nearctaphis bakeri*, an effective vector of SbDV. SbDV was transmitted first from clover to soybean by *N. Bakeri*, and then serially passaged on soybean by either *N. bakeri* or *A. glycines*. Transmission efficiency for *N. bakeri* decreased from 33% to 20% over 6 serial transmissions on soybeans, and then transmission ceased. Transmission efficiency by *A. glycines* varied from 6–20%, ceasing after one or two transmissions. Although transmissibility was lost, real time RT-PCR indicated that virus titers increased in soybeans with each sequential transmission by both vectors. Sequence analysis following the last passages of SbDV in soybeans identified 5 non-synonymous mutations in the non-transmissible isolates compared with *A. glycines* and *N. bakeri* transmissible isolates. These mutations are likely related to SbDV adaptation to soybeans and the loss of aphid transmissibility.

Viruses infecting soybean (*Glycine max* L. Merill) in Nigeria
I. TIME (1), G. I. Atiri (2), P. Lava Kumar (3)
(1) International Institute of Agriculture and University of Ibadan, Ibadan, NIGERIA; (2) Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, NIGERIA; (3) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA
Phytopathology 100:S126

Nigeria is the largest producer of soybean in Africa with over 600,000 ha devoted to the crop production. A survey for soybean virus diseases was conducted in all the major soybean producing regions in mid altitude, guinea savanna and derived savanna agroecological zones of Nigeria. A total of 1017 soybean leaf samples were collected from 135 locations and they were analyzed for *Bean pod mottle virus* (BPMV), *Black eye cowpea mosaic virus* (BlCMV), Cowpea aphid-*borne mosaic* (CABMV), *Cowpea mottle virus* (CoMV), *Cowpea mosaic virus* (CoMV), *Cucumber mosaic virus* (CMV), *Cowpea severe mosaic virus* (CPSMV), *Cowpea mild mottle virus* (CPMMV), *Southern bean mosaic virus* (SBMV), *Tobacco streak virus* (TSV) and *Soybean mosaic virus* (SBMV) using enzyme-linked immunosorbent assay (ELISA). Viruses were detected in 77% of the samples and all the eleven viruses were detected in soybean. Viruses were detected in all the agroecological zones and the frequency of infection was highest in derived savanna (83%), followed by mid-altitude (78%) and guinea savanna (72%). CPMMV was the most prevalent virus detected in 72% of the total samples. SMV, BICMV, CMV and BPMV were detected in 20%, 21%, 7% and 2% of the samples, respectively. Fifteen combinations of mixed virus infections were detected. CPMMV was common in every combination and it was also detected in 17% of the asymptomatic samples. CPSMV, detected in two locations, was the first record of its occurrence in soybean.

Centipedegrass: A new host of *Colletotrichum sublineola*
M. TOMASO-PETERSON (1)
(1) Mississippi State University, Mississippi State, MS, U.S.A.
Phytopathology 100:S126

Centipedegrass (*Eremochloa ophiuroides*) is rapidly becoming the home lawn turf of choice in Mississippi. It is a medium-textured, slow-growing warm-season turfgrass that requires low input, is well-adapted to a wide range of soil conditions but grows best in sandy, acidic soils of low fertility and can withstand some shade. Diseases arise when centipedegrass is over-managed with fertility and irrigation inputs and low height of cut. In spring of 2007, centipedegrass home lawns exhibited symptoms of leaf chlorosis and sheath blight, and irregular, chlorotic patches associated with thinning turf. Hyphal appressoria, diagnostic of *Colletotrichum*, were observed in leaf sheath tissue of diseased plants. Pure cultures of the fungus on potato dextrose agar produced grey mycelia surrounded by a bright yellow exudate; falcate conidia and globose to irregular hyphal appressoria were abundant. Phylogenetic analysis using Apn2, Mat1, Sod2 and ITS sequence data identified the fungus as a distinct lineage of *C. sublineola*, with centipedegrass isolates clustering separately from isolates from sorghum and johnsongrass. Koch's postulates were performed in the growth chamber by inoculating seedlings with a spore solution of *C. sublineola*. Chlorosis was observed 10 dpi; hyphal appressoria were observed 21 dpi. The pathogen was successfully re-isolated from

diseased tissue. A new host of *C. sublineola* and a new disease of centipedegrass have been identified.

Transcriptome analysis of a susceptible *Glycine max-Phakopsora pachyrhizi* interaction using next generation sequencing

A. TREMBLAY (1), P. Hosseini (2), N. W. Alkharouf (2), S. Li (3), D. G. Luster (4), B. F. Matthews (1)
(1) USDA ARS PSI, Beltsville, MD, U.S.A.; (2) Towson University, Towson, MD, U.S.A.; (3) USDA ARS CGPRU, Stoneville, MS, U.S.A.; (4) USDA ARS FDWSR, Frederick, MD, U.S.A.
Phytopathology 100:S127

Soybean is in the top five agricultural products in the United States. Soybean rust (SR) is caused by an exotic obligate fungus. We want to analyze the expression pattern of SR and its soybean host genes during the infection. Thus, libraries were constructed from different soybean cells infected by SR at different time-points and sequenced using a Solexa platform. Infection sites were visualized by immunofluorescence and isolated by laser capture microdissection. DNA sequences were aligned to the soybean genome and homology searches were conducted to identify genes. From sequences without similarity to soybean genome, contigs were formed and homology searches were conducted. All time-points give us a limited number of sequences aligning to the soybean genome (3,330 sequences/time-point). However, we found much more sequences (9,000,000) without any homology to the soybean genome. These are expected to be SR sequences. Contigs build from those sequences had homology with genes involved in fungal development, lignin degradation, signal transduction and intracellular communication (chitin deacetylase, glyoxal oxidase, serine threonine protein phosphatase, transthyretin). We also found contigs containing signal peptide which are common to fungal virulence factor and others containing catalase and peroxidase domain which are involved in defense. Target pathogen as well as some relevant host genes will be studied to determine if they can be used to control SR in soybean.

Sequence data of *Xanthomonas* strains isolated from U.S. rice fields reveals substantial divergence from *Xanthomonas oryzae* pvs. *oryzae* and *oryzicola*

L. R. TRIPLETT (1), J. P. Hamilton (2), N. A. Tisserat (1), C. R. Buell (2), J. E. Leach (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.; (2) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S127

Xanthomonas oryzae pathovars *oryzae* (*Xoo*) and *oryzicola* (*Xoc*) are the causal agents of bacterial leaf blight and bacterial leaf streak of rice, respectively. *Xoo* causes significant losses in Asia, and is a select agent organism subject to international quarantines. Two decades ago, *Xanthomonas* spp. were isolated in the southern United States from rice plants displaying water-soaked, chlorotic lesions. Serological studies suggested that these isolates were *Xoo*, despite a weak virulence phenotype and distinct RFLP profiles. In this study, short-read sequencing technology was used to sequence the genomes of two *Xanthomonas* strains isolated from rice in Texas and Louisiana. Sequence reads were assembled into contigs for comparative genome analysis. Phylogenetic analysis based on *hrp*, *gum*, and *rpf* clusters and housekeeping genes revealed a close relatedness among the U.S. strains, but substantial genetic divergence between the U.S. strains and Asian strains of *Xoo* and *Xoc*. Reciprocal Blast alignment of predicted coding sequences supported this observation. The genome sequence revealed a lack of TAL effectors and Xoo8-family transposons in the U.S. strains, as well as the presence of novel putative gene clusters. These results support the hypothesis that U.S. strains of *Xanthomonas* belong to a distinct subgroup or subspecies of *X. oryzae*, likely introduced in a single event prior to the divergence of *Xoo* and *Xoc*. Diagnostic primers were developed to distinguish the U.S. strains from *Xoo* and *Xoc*.

Characterization of salicylate hydroxylase of "*Candidatus* Liberibacter asiaticus" and its role in plant defense suppression

P. TRIVEDI (1), N. Wang (1)
(1) Citrus Research and Education Center, University of Florida/IFAS, Lake Alfred, FL, U.S.A.
Phytopathology 100:S127

Citrus huanglongbing (HLB), associated with pathogen *Candidatus* Liberibacter asiaticus (Las), is a devastating disease to the U.S. citrus industry. It is intriguing to gain knowledge on the mechanism(s) by which Las evades host defense responses. One orf encoding a homolog of salicylate hydroxylase (*sahA*) is present in the genome of Las. Salicylate hydroxylase is responsible for salicylic acid (SA) breakdown. SA is important for basal defense, hypersensitive response, and systemic acquired resistance. We postulate that salicylate hydroxylase of Las is involved in suppressing host defenses against pathogen infection. To test this hypothesis, two set of experiments were performed to determine (a) function analysis of sahA, and (b) effect of Las infection on expression of defense related gene (*PR-1*) using QRT-PCR. We first expressed *sahA* in *Escherichia coli* and found that salicylate hydroxylase of Las is functional and can breakdown various salicylate based substrates into catechol. To determine expression level of defense related genes after Las infection, *Xanthomonas axonopodis* pv. *citri* strain A^W (Xac A^W) was used to induce *PR-1* gene expression. The *PR-1* gene expression in Xac A^w challenged plants which were inoculated previously with Las was lower than Xac A^w challenged healthy plants. Our data suggest that modulation of SA production and subsequent regulation of defense related genes such as *PR-1* gene could be one of the mechanisms deployed by Las to evade plant defense responses.

Molecular diversity and population dynamics of *Xanthomonas axonopodis* pv. *manihotis* in the Caribbean region of Colombia

C. A. TRUJILLO (1), S. Restrepo (1), C. E. López (2), J. Esquivel (3), A. Bernal (1)
(1) Universidad de Los Andes, Bogota, COLOMBIA; (2) Universidad Nacional de Colombia, Bogota, COLOMBIA; (3) Corporation for the Sustainable and Participative Management of the Rural Small Growers in Colombia, PBA, Bogota, COLOMBIA
Phytopathology 100:S127

Cassava bacterial blight is the most important bacterial disease in this crop. Previous population studies of *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), the causal agent of this disease, showed a prominent diversity index, with pathogen migration being an important determinant of diversity among regions. Ten years later, aiming at establishing the current status of population structure of *Xam* in this region, different agroecological zones were selected. Bacterial samples were collected in five field trips from September of 2008 to November of 2009. Bacterial haplotypes were determined by AFLPs and clustering analysis established relationships among them, as well as their distribution on the agroecological regions. Additionally, effector genes were sequenced to determine their degree of variability and to assess the presence and nature of selection exerted by the host. The results confirmed a prominent diversity index and haplotype migration through the months in the Caribbean region. This could be due to deficient cultural practices and/or differences in the environmental conditions of the regions. On the other hand, a low variability was detected on effector genes. This study shows the current condition of populations of Xam in the Caribbean region of Colombia and it contributes to improve the existing bacterial blight control practices.

The use of Xspecies microarray to study changes in gene expression in phytoplasma-infected *Catharanthus roseus*

M. G. TUFFEN (1)
(1) University of Nottingham, Loughborough, UNITED KINGDOM
Phytopathology 100:S127

Phytoplasmas are specialised plant pathogenic bacteria that are responsible for several economically important diseases of crops. They are spread by sap sucking insects such as leafhoppers and within the plant are limited to the phloem; they can not be cultured in vitro. *Catharanthus roseus*, or Madagascar periwinkle, is used an experimental host for phytoplasmas as it is susceptible to many phytoplasma strains and gives a good range of representative symptoms. The Xspecies microarray technique allows a microarray to be used on a species it was not designed for. In this study, gene expression was compared between healthy and sweet potato little leaf phytoplasma infected *Catharanthus roseus*, which shows symptoms of dramatically reduced leaf size, chlorosis and increased branching. Sixty-six differentially regulated genes were identified, including up regulation of auxin related genes and a decrease in the expression of photosynthesis related genes. A number of these identified genes have subsequently been isolated from *C. roseus* and quantitative PCR has been used to confirm the expression results.

Investigations of crown gall in the commercial propagation of weeping fig

W. TURECHEK (1)
(1) USDA ARS SAA SPP, Fort Pierce, FL, U.S.A.
Phytopathology 100:S127

Agrobacterium larrymoorei causes galls on the trunk, branches, and stems of weeping fig (Ficus benjamina L.). The extent to which this pathogen is transmitted through cuttings and the extent to which it is spread through the mother planting as a result of preparing air layers and subsequently pruning them to produce braided plants were studied in a commercial nursery. Branches selected for propagation were chosen from mother trees with no visible signs of galls and tagged for future tracking. Rooted branches were cut from the mother tree, braided with 2 to 4 other branches, planted in pots, and then placed on ground cloth to establish. Gall formation was tracked on all branches of each braided plant. Additional ratings were taken in the mother tree planting 6 months after pruning. In the mother tree planting, there was significant spatial correlation between mother trees infected before pruning

and trees infected after pruning. There were also significant correlations between infected mother trees and braided plants established with 1 or more branches propagated from infected mother trees. There did not appear to be any correlation between the time of year when plants were propagated and gall formation. Although pruning shears are routinely soaked in sterilization medium in commercial practice, the degree of sterilization achieved in this nursery was not sufficient for reducing disease spread.

Supplementing nutrition with calcium and potassium silicate to control *Botrytis cinerea* in poinsettia stock plants

L. TUROOP (1), J. E. Faust (2)
(1) Jomo Kenyatta University of Agric & Technology, Nairobi, KENYA; (2) Clemson, SC, U.S.A.
Phytopathology 100:S128

Root application of silicon (Si) on poinsettia stock plants was evaluated for its effects in reducing *B. cineria* and promoting cutting performance in storage and propagation. Fertilization of two poinsettia varieties raised on soilless media was supplemented with calcium or potassium silicates at the concentrations of 125, 250 and 500 ppm. Control plants were drenched with same amount of distilled water. leaf tissue were inoculated with a spore suspension and the development of botrytis was monitored. Shelf life and performance of cuttings in propagation was assessed. Calcium and potassium silicate significantly reduced the severity and incidence of *B. cineria* on Si treated plant tissue compared to the control. Applying 500 ppm Si reduced the disease severity by up to 27.8% and disease incidence by 33.1%. The losses associated with botrytis in storage were reduced by >30% in high Si fertilized plants. Performance and incidence of botrytis in propagation was significantly influenced by Si application. Although infection occurs on tissue treated with high Si rate, lesion expansion was significantly reduced. The net effect of Si on poinsettia stock plants is an overall reduction in disease development, by eliciting a defense reaction as indicated by a significant increase in total tissue phenolic content with increase in Si rate, thereby slowing the infection rate of *B cineria*.

Influence of environmental conditions on the development of soybean rust epidemics in soybean fields in Nigeria

M. TWIZEYIMANA (1), P. S. Ojiambo (2), R. Bandyopadhyay (3), G. L. Hartman (1)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.; (3) International Institute of Tropical Agriculture (IITA), Ibadan, NIGERIA
Phytopathology 100:S128

Epidemics of soybean rust (*Phakopsora pachyrhizi*) and environmental factors that favor rust development were studied under natural infection in Nigerian soybean fields from 2004 to 2006 by sequentially planting early (TGx 1485-1D) and medium (TGx 1448-2E) maturing soybean cultivars at intervals ranging from 1 to 1½ months. Disease onset and final disease severity varied substantially among all planting times. Little or late infection was observed on soybeans planted during the dry season (November to March), while disease severity was high and rate of progress much faster on soybeans planted during the wet season (April to October). Across years, disease severity was consistently higher on soybeans planted in August. Infection and disease development were significantly ($P < 0.05$) affected by environmental factors with strong correlations between rust severity and evaporation ($r = –0.726$ and –0.632) and wind speed ($r = –0.708$ and –0.634) for TGx 1485-1D and TGx 1448-2E, respectively and slight correlations between disease severity and solar radiation, maximum and average temperatures for both cultivars. Strong and slight correlations were observed between urediniospores concentration (spores/m^3 of air) and weather parameters. There was a significant ($P < 0.05$) positive correlation ($r = 0.700$) between rust severity and urediniospores concentration. Based on this study, soybean growers in Nigeria need to modify their planting date slightly to reduce rust epidemics.

Epidemiology of two *Diodia virginiana* criniviruses

I. E. TZANETAKIS (1), A. Cortez (2), J. Zhou (1), B. Poudel (1), L. Hladky (2), R. Larsen (3), W. M. Wintermantel (2)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) USDA-ARS, Salinas, CA, U.S.A.; (3) Vegetable and Forage Crops Research Laboratory, USDA-ARS, Prosser, AR, U.S.A.
Phytopathology 100:S128

The genus *Crinivirus*, family *Closteroviridae*, includes several emerging viruses that cause disease in crops around the world. *Diodia virginiana*, family *Rubiaceae*, a widespread noxious weed of the southeastern United States, is known to be infected by a crinivirus, *Diodia vein chlorosis virus* (DVCD). During the original characterization of DVCV, herbaceous hosts were tested as alternate hosts for the virus but none of the major crops of the Southeast was included in that study. There are no detection tests available for DVCV and given the emergence of several criniviruses, we began developing detection tests for the virus and tested the possibility that crops in the Southeast could be infected with the virus. During the molecular characterization process, we determined that there were two criniviruses infecting Diodia, both transmitted by the greenhouse and banded winged whiteflies (*Trialeurodes vaporariorum* and *T. abutilonea*, respectively). The Diodia viruses belong to Crinivirus group 1, along with the small fruit-infecting criniviruses and Potato yellow vein virus. The similarity of the diodia viruses with the small fruit-infecting criniviruses probed us to test the possibility that the two diodia viruses can infect Rosaceae hosts, especially blackberry, a crop affected by two criniviruses that can synergistically cause blackberry yellow vein disease.

Protecting the endangered *Eugenia koolauensis* from further loss in the Hawaiian Environment

J. UCHIDA (1), C. Y. Kadooka (1), R. Hauff (2), L. Loope (3)
(1) University of Hawaii, Honolulu, HI, U.S.A.; (2) State of Hawaii Department of Land & Natural Resources, Division of Forestry & Wildlife, Honolulu, HI, U.S.A.; (3) USGS Pacific Island Ecosystems Research Center, Haleakala Field Station, Makawao, HI, U.S.A.
Phytopathology 100:S128

The endangered *Eugenia koolauensis* exists in Hawaii as only about 200 plants, scattered in small areas on two islands. The population has been at high risk from recurrent fires, wild boars, and environmental degradation. But with fencing and identification of the few remaining plants, conservation biologists were hopeful that this plant could be saved. In 2005, a new invasive rust, *Puccinia psidii*, the guava rust, was found attacking ohia (*Metrosideros polymorpha*,) at a commercial nursery. A quick survey of the surrounding forests, showed that it was on rose apple (*Syzygium jambos*,) and other trees, as this rust attacks many in the Myrtaceae. It was also found on E. koolauensis. Rose apple is an introduced species and is now widely distributed on all major islands. Young leaves are severely blighted, turn black, and defoliate. New leaves are then produced and the same cycle occurs. Soon the reserves of the tree are used and many trees are now dying. Unfortunately, E. koolauensis, suffers the same fate. Three fungicides were tested on ohia at a commercial nursery and myclobutanil was identified as effective in protecting young leaves. This product was taken to the field and tested on two plants with good efficacy observed. Expanded efficacy and phytotoxicity testing will be completed. Experimental studies for registration of this compound for use in the forest, are being planned.

Biological control of *Macrophomina phaseolina* on sunflower in Pakistan

H. ULLAH (1)
(1) Dept. of Plant Pathology, University of Agriculture, Pakistan
Phytopathology 100:S128

In vitro sensitivity of *Macrophomina phaseolina* to potentially antagonistic fungi was determined on potato dextrose agar. *Aspergillus flavus* was the most effective reducing growth of *M. phaseolina* by 66%, followed by *Aspergillus niger* (55.6%), *Trichoderma viride* (51.1%), *Trichoderma harzianum* (26.7%) and *Penicillium capsulatum* (11.1%) respectively, Seeds of four sunflower varieties (G-66, HRSB1, G-72, G-51_ were treated with cultures of *A. flavus*, *A. niger*, *T. viride* and *P. capsulatum* and combinations.suppression by *A. niger*, *P. capsulatum* and *T. viride* was 64.9, 63.8 and 31.89%, respectively. The decrease in disease incidence compared with controls was 100% when seed were treated with a combination of *A. niger* and *A. flavus*, whereas the *A. niger* and *T. viride* combination reduced disease by 30.8%. Antagonist combinations containing *A. flavus* and *A. niger* or (81.41%) *A. flavus* and *P. capsulatum* reduced disease by 81.4 and 27.33%, respectively, compared with controls.

Evaluation of varying sugar concentrations for growth of and aflatoxin production by *Aspergillus flavus*

S. UPPALA (1), K. L. Bowen (1)
(1) Dept. Entomology and Plant Pathology, Auburn University, AL, U.S.A.
Phytopathology 100:S128

Aflatoxin contamination by *Aspergillus* spp. is one of the main factors affecting peanut quality. Available carbon source is one of the nutritional factors affecting aflatoxin production. Abundant aflatoxin production is generally associated with substrates containing elevated levels of specific carbohydrates. When peanuts are subjected to drought and temperature stress, carbohydrate content of peanut seed is higher than in non-stressed seed. Glucose, fructose and sucrose are generally accepted as the carbohydrates that induce aflatoxin formation by *A. flavus* and these are primary components of peanut seed. Our objective is to evaluate varying sugar concentrations, in the amounts present in peanut seed, for their effect on the growth of and aflatoxin production by *A. flavus*. We have grown *A. flavus* in media containing different amounts of sugars for limited periods of time. Aflatoxins were analyzed and fungal mycelial weights were determined. Mycelial weight

increased significantly with increases in sugar concentration. Other parameters such as total aflatoxin and aflatoxin B1 are low at low sugar levels and are slightly greater when sugar content is slightly higher as found in drought-stressed compared to non-stressed peanut seed. Toxin per gram of mycelium is usually high at low sugar levels.

Understanding the nonhost resistance mechanisms of *Medicago truncatula* to Asian soybean rust and switchgrass rust

S. UPPALAPATI (1), Y. Ishiga (1), S. Mittal (1), H. Schultheiss (2), K. S. Mysore (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.; (2) BASF Plant Science Company GmbH, Limburgerhof
Phytopathology 100:S129

Asian soybean rust (ASR) of soybeans caused by *Phakopsora pachyrhizi* is a major concern and there is an urgent need for identification of durable resistance to ASR. Switchgrass rust caused by *Puccinia emaculata* is a also growing concern for bioenergy crop production. We found that *Medicago truncatula*, a model legume, displays nonhost resistance to *P. pachyrhizi* and *P. emaculata*. Initial characterization of the *M. truncatula-P. pachyrhizi* incompatible interaction has shown that while the fungus forms long germ-tubes and directly penetrates M. truncatula epidermal cells resulting in small necrotic lesions, it fails to sporulate on *M. truncatula*. Analysis of global transcriptional changes during *M. truncatula-P. pachyrhizi* interactions identified several up-regulated genes involved in phytoalexin biosynthesis and PAMP-triggered defense responses during nonhost resistance. Interestingly, the M. truncatula nonhost response to P. emaculata was not associated with major transcriptional changes in phenylpropanoid pathway or pathogenesis related genes. In addition, a reverse/forward Tnt1 insertional mutant screen identified an enhanced penetration mutant that was more susceptible to *P. pachyrhizi*, but not to *P. emaculata*. However, another resistant to rust (rer) mutant showed resistance to both the nonhost pathogens. These results suggested that *M. truncatula* possibly employs various nonhost resistance mechanisms to different nonadapted rust fungi.

Etiology of branch dieback of olive trees (*Olea europaea* L.) in California

J. R. URBEZ-TORRES (1), F. Peduto (1), W. D. Gubler (1)
(1) University of California, Davis, Davis, CA, U.S.A.
Phytopathology 100:S129

Branch dieback of olive trees as consequence of perennial canker formation in the vascular tissue is a major concern among growers due to significant economical losses in mature olive orchards as a result of both yield reduction and increase of production costs. However, the importance that branch dieback has in olive health in California has not yet been evaluated. Therefore, field surveys were conducted throughout the main olive-production regions to determine the incidence of branch dieback in California. Declining branches and trunks were collected in order to identify the fungal pathogens associated with perennial cankers and subsequent dieback of olive trees. In all, over 700 samples were collected from 60 different olive orchards throughout California. Olive branch dieback was observed in all mature orchards surveyed in California. Isolation of samples showing perennial cankers followed by morphological studies and phylogenetic analyses of three loci allowed us to identified 16 different fungal species in the families Botryosphaeriaceae, Valsaceae, Diatrypaceae, Coriolaceae, and Schizophyllaceae to be associated with olive cankers in California. In order to determine the role that these fungi play on olive health in California, pathogenicity tests were conducted in Sevillano and Manzanillo cultivars. Extent of vascular discoloration and percentage of recovery from inoculated wounds showed all fungi to be pathogenic; however, virulence varied by species.

Evaluation of rhizobacterial strains as *Fusarium oxysporum* biological control agents

R. Urrea (1), L. F. Cabezas (2), S. Restrepo (2), P. JIMENEZ (1)
(1) Univ Militar Nueva Granada, Bogota, COLOMBIA; (2) Univ de Los Andes, Bogotá, COLOMBIA
Phytopathology 100:S129

Different reasons had stimulated the development of agro products based in microorganisms and their metabolism. This has stimulated the screening of many sources of microorganisms, among them the rhizosphere. On the other side, *Fusarium oxysporum* is an important pathogen to many crops, demanding its control for huge amounts of fungicides that are becoming useless controlling the fungus. Since *Physalis peruviana* is an important crop, and *F. oxysporum* is its more limitant pathogen, rhizobacteria from *P. peruviana* rhizosphere were collected in 4 production areas in Bogota plateau, Colombia. In two screening processes 5 bacteria, out of 150, were chosen because their ability to reduce by 50% the *in vitro F. oxysporum* radial growth, and reduce the number of macro and micro conidia per colonial area. These bacteria, 2 isolates of *Bacillus subtilis*, 2 of *Pseudomonas flourecens* and 1 of *Pseudomonas* sp. were also evaluated by their effect on plant growth and reducing the amount of disease in planta. Their effect on germination was not a clear one, but they increased the dry weight by 40%, and lowered the AUDPC by about 70%. At present these 5 bacterias have been incorporated in different schemes of evaluation with different pathogens.

Identification of seedling pathogens from soybean planted in field soils at three temperatures

K. URREA (1), J. Rupe (1), C. Rothrock (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S129

Seedling diseases are caused by a number of pathogens that can work singly or as a complex. To identify the primary seedling pathogens of soybean in Arkansas, soil was collected from two sites (Hope and Stuttgart) at three planting dates (April, May and June). These soils were planted with seeds of three soybean cultivars, each of which had one of seven fungicide seed treatments. Tests were conducted in growth chambers at 21°C (April), 25°C (May) or 28°C (June). After 18 to 26 days, isolations were made from the roots and seeds on water agar and isolates were identified to species. *Pythium* spp. and *Fusarium* spp. were the most frequently isolated pathogens at each temperature with *P. sylvaticum* (65%) and *F. oxysporum* (45%) being the predominate species. *Rhizoctonia solani* was 8% of the isolates. *Pythium* spp. and *R. solani* were more frequently recovered from soil from Hope than Stuttgart. Recovery of *Pythium* spp. and *Fusarium* spp. was greatest for the May planting date followed by June and April. Recovery of *R. solani* was higher in April and May and less in June. Over 90% of *P. sylvaticum* isolates were highly virulent to soybean while the percentage of virulent *F. oxysporum* isolates was greatest at 28°C (93%) followed by 25°C (79%) and 21°C (57%). Only recovery of *R. solani* was affected by seed treatment. These results show that *P. sylvaticum* and *F. oxysporum* are the major components of a dynamic seedling disease complex in Arkansas.

A breakthrough in the field of agriculture

K. VADIVEL (1)
(1) Annamalai University, Chidambaram, INDIA
Phytopathology 100:S129

Mung bean (*Vigna radiata* L. Wilczek) commonly known as green gram is the third important grain legume in India. Natural products like lime, hydrated lime, turmeric powder and livestock excrements have been tested by seed treatment and spraying them on ADT.3 green gram variety15 times at biweekly intervals in two rabi seasons (Dec – March 2008 and 2009) and reported. Under *in vitro* studies Annamalai mixture (cow urine +cow dung +sheep dung +poultry litter+neem cake) 50% concentration recorded the maximum vigour of 2400 followed by turmeric powder 1900 whereas in control it was 280. Under pot trial, the Annamalai mixture recorded the maximum yield of 11.4 g/plant whereas in field experiment T2 (cow urine +cow dung) recorded the maximum of 3.3 tonnes/ha followed by Annamalai mixture 3.25 tonnes whereas in control it was 193.1 kg/ha only. The fungal diseases like cercospora leaf spot and rust infection was minimum in Annamalai mixture whereas lime and turmeric powder recorded no powdery mildew infection. Similarly, yellow mosaic disease was totally absent in turmeric powder and livestock excrements applied treatments. Various nutrients and plant growth promoters present in natural products might have influenced the plant growth and increased the yield of green gram manifold and the antimicrobial properties like ammonia, silica, antioxidants like curcumin and calcium reduced the disease incidence. Thus, use of such a simple as well as the traditional method may open up a new chapter in agriculture.

Initial characterization of a *Xanthomonas* sp. causing bacterial spot of shrub rose (*Rosa* spp.)

G. VALLAD (1), C. Summers (1), H. Adkison (1), E. Margenthaler (1)
(1) University of Florida, Wimauma, FL, U.S.A.
Phytopathology 100:S129

A severe bacterial spot of shrub rose (*Rosa* spp.) caused by a xanthomonad was observed during summer production in Florida. Foliar symptoms consisted of small black lesions with defined margins that were fairly vein delimited and often located along leaf margins. Based on fatty acid composition and 16S rRNA sequence, the strain was most closely related to several pathovars of *Xanthomonas axonopodis*. The 16S-23S rRNA intergenic spacer (ITS) and flanking portions of the 16S and 23S rRNA genes were sequenced and compared among three rose strains and those of several characterized strains of *X. citrumelo*, *X. euvesicatoria*, *X. dieffenbachiae*, *X. manihotis*, and *X. perforans*. Sequence identity within the nearly 2 kb region was greater than 98.3% among all strains with 100% identity among the rose strains. The rose strains exhibited 99.9% identity with *X. perforans*. Phylogenetic analyses of the ITS region consistently grouped rose strains closest to *X. perforans*. Rose strains caused few symptoms when infiltrated at

10^6 cfu/ml into leaves of citrus, tomato, pepper, or several members of the Euphorbiaceae or Araceae. However, strains were clearly pathogenic on rose and another Rosaceae, Indian Hawthorne (*Rhaphiolepis indica*). Results suggest that the rose strains may represent a new species or subspecies of *Xanthomonas*. Further characterization of rose strains through multi-locus sequence typing and host testing is in progress. This is the first pathogenic *Xanthomonas* sp. associated with rose.

A rapid screening method for fungicide resistance in *Alternaria alternata*
B. VEGA (1), P. F. Harmon (2), M. M. Dewdney (1)
(1) CREC, University of Florida, Lake Alfred, FL, U.S.A.; (2) Department of Plant Pathology, University of Florida, Gainesville, GA, U.S.A.
Phytopathology 100:S130

Alternaria brown spot (ABS) of tangerines and tangerine hybrids is a very important foliar disease affecting leaves, twigs and young fruit, rendering them unsalable. Strobilurin fungicides have been used for ABS control for many years and are the most effective products registered. In the last two years, reduced ABS control has been observed in Florida groves with strobilurin applications. Preliminary studies found strobilurin resistant populations, and a larger study was initiated. The traditional method of fungicide resistance evaluation employs media amended with logarithmically-diluted fungicide concentrations where the mycelium growth or germ tube length of monosporic isolates are evaluated. The incorporation of an oxidation-reduction indicator, resazurin, to measure spore respiration in a microtiter assay could significantly reduce cost and time. We evaluated a minimal media, a complete media and potato dextrose broth (PDB) amended with calcium carbonate in order to optimize this assay. The greatest percent reduction of resazurin was observed in PDB and complete media. At 10^5 spores/ml, the assay sensitivity was optimal for fungicide resistance evaluation. Alternative respiration was suppressed with the addition of SHAM to the strobilurin assays. Isolates had comparable ED_{50} values for both methods. Sensitive and baseline isolates were lower than 0.79 µg/ml of azoxystrobin while resistant isolates had values greater than 10 µg/ml.

Electroporetic Potyvirus transfection of pepper protoplasts
N. Y. VELASQUEZ (1), S. Suh (1), J. F. Murphy (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S130

Potyviruses are a persistent threat to bell pepper (*Capsicum annuum* L.) production worldwide. Three Potyviruses commonly detected in diseased pepper plants are *Potato virus Y* (PVY), *Pepper mottle virus* (PepMoV) and *Tobacco etch virus* (TEV). We have studied pepper resistance responses to Potyvirus infection at the cellular level using freshly isolated protoplasts and a polyethylene glycol (PEG) inoculation procedure. The PEG procedure poses difficulties with experiment to experiment precision. We therefore developed an electroporation inoculation procedure for Potyviruses using a Bio-Rad Gene Pulse Xcell. Experiments evaluated voltage, number of pulses, time interval between pulses, viral RNA concentration and number of protoplasts inoculated. For each test, virus accumulation was determined by ELISA and protoplast viability was monitored. Consistent infection with the highest virus titer and protoplast viability resulted when 40 µg virus RNA was used to inoculate 500,000 protoplasts using two 25-msec pulses of 200 volts each with a 10-sec time interval between pulses.

Molecular characterization and ELISA based detection of Bean leafroll virus and Pea enation mosaic virus from the Pacific Northwestern U.S.A.
B. Vemulapati (1), K. L. DRUFFEL (1)
(1) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S130

The complete genomic sequence of one Pea enation mosaic virus (PEMV) isolate from Idaho (PEMV-ID) and one Bean leaf roll virus (BLRV) isolate from Washington State (BLRV-WA) were determined. PEMV-ID contained five ORFs. ORF4, which encodes the CP, shared a maximum identity of 98.9% with other members of PEMV while the least amino acid identity was seen with ORF-1. Phylogeny tree based on CP sequences showed that PEMV-ID grouped with PEMV isolates UP58 and Germany. BLRV-WA contained five ORFs and ORFs 1 and 3 shared a maximum identity of 99.4% with respective ORFs of known BLRV isolates, while the least was with ORF-2. Antigen-coated plate (ACP) ELISA assays for the detection of BLRV and PEMV were developed using antisera raised against recombinant coat proteins (CP) of BLRV and PEMV, respectively. Pea and alfalfa samples collected from different fields in Washington and Idaho were tested. BLRV antiserum detected the virus in up to 1:3200 dilution of infected samples, and anti-PEMV serum could detect the virus in pea leaf extracts up to 1:6400 dilution.

Recombinant antibody-mediated multiple disease tolerance in canola
S. S. VERMA (1), W. Yajima (1), M. H. Rahman (1), S. Shah (2), Y. Liang (1), N. N. Kav (1)
(1) University of Alberta, Edmonton, AB, CANADA; (2) Alberta Innovates, Vegreville, AB, CANADA
Phytopathology 100:S130

Plants are constantly exposed to numerous potential pathogens endowed with diverse modes of attack and have the potential to cause significant yield reduction, up to 15%, due to pathogen attack. Earlier research has shown that external application of polyclonal and monoclonal antibodies in plants can impart tolerance against certain bacteria and fungi. This approach has recently been improved further by introducing cDNA encoding recombinant antibodies (rAbs) into plants. We have generated transgenic lines of Brassica napus canola expressing anti- Sclerotinia sclerotiorum ScFv and ScFv-fusion proteins, and several of these transgenic lines expressing those constructs exhibited increased tolerance to multiple diseases. Especially, we observed that these transgenic B. napus were tolerant against Sclerotinia sclerotiorum, Alternaria brassicae and Leptosphaeria maculans all of which causes major diseases in canola. In addition, another construct containing cDNA encoding ScFv fused with a known anti-microbial peptide increased the tolerance of transgenic canola against these pathogens even further. Advantages of this approach include the fact that recombinant antibodies can be engineered against almost any target molecule, and it has been demonstrated that the expression of functional pathogen-specific ScFv's in plants can confer effective pathogen protection.

Genome organization and structure of a putative member of the *Flexiviridae* family infecting sweet cherries
D. V. VILLAMOR (1), K. L. Druffel (2), K. Eastwell (1)
(1) Washington State University, Prosser, WA, U.S.A.; (2) Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S130

A novel disease was observed in an isolated sweet cherry (*Prunus avium*) orchard in Washington State. The graft transmissible agent induced foliar symptoms on cultivar 'Bing' that were reminiscent of cherry rusty mottle disease, but also induced severe stem pitting on Montmorency interstock. Because of this distinct symptomatology, the putative causal agent is herein referred to as Montmorency stem pitting virus (MMSPV). Electrophoresis of double-stranded RNA isolated from the infected tissue revealed a product of ca. 8.5 kbp supporting a viral etiology. A primer pair derived from a cloned fragment of the dsRNA yielded amplicons of the anticipated size from trees with MMSPV symptoms, but not from trees infected with other common viruses known to infect sweet cherry trees. Primers designed from the 3′-terminus of foveaviruses yielded amplification products of 1.1 kbp that were cloned and sequenced. The amplicon contained a putative coat protein sequence that shared 74.2% sequence identity to the published sequence of *Cherry green ring mottle virus* and 75.7% sequence identity to *Cherry necrotic rusty mottle virus*, both unassigned viruses of the *Betaflexiviridae* family. Primers designed from sequences obtained by 5′ and 3′ RACE allowed the complete genome of ca. 8.7 kb to be amplified and cloned. Sequencing of the complete virus genome is currently underway.

Disease management in strawberry production: The Philippine experience
L. M. VILLANUEVA (1)
(1) Benguet State University, La Trinidad, Benguet, PHILIPPINES
Phytopathology 100:S130

Strawberry is grown only in Benguet Province because of its unique climatic conditions. It has been a lucrative source of income for Benguet farmers and adds to the revenue of Benguet Province. Diseases caused by fungi, bacteria and nematodes are important limiting factors in strawberry production in the area. However, researches on strawberry diseases are very nil which could be attributed to limited funding support to the crop. To identify the major diseases, soil and plant samples were collected from the strawberry growing areas in Baguio City and Benguet Province. The following diseases were observed: verticillium wilt, red stele, leaf scorch, leaf spot, leaf blight, gray mold and other minor disorders. On the other hand, the root lesion and strawberry crimp nematodes were the economically important nematode pests identified. Six strawberry cultivars namely Sweet Charlie, Camarosa, Festival, Whitney Earlibrite, and Winterdawn were evaluated for their resistance to the major fungal pathogens and root lesion nematode. Sweet Charlie, the most preferred strawberry cultivar seems to be resistant to some fungal pathogens and root lesion nematode. Other potential disease management options tested include the following: use of indigenous mulching materials, rotation with broccoli, use of biocontrol agents and application of baking soda. Integration of effective and compatible disease management strategies would be very crucial in the improvement of the strawberry industry in the region.

Detection of viable *Phakopsora pachyrhizi* spores by indirect immunofluorescence and propidium iodide staining

R. VITTAL (1), J. S. Haudenshield (2), G. L. Hartman (3)
(1) University of Illinois - Urbana Champaign, Urbana, IL, U.S.A.; (2) USDA-Agricultural Research Service, Urbana, IL, U.S.A.; (3) University of Illinois - Urbana Champaign; USDA Agricultural Research Service, Urbana, IL, U.S.A.
Phytopathology 100:S131

We developed a rapid and reliable technique for the detection of viable *Phakopsora pachyrhizi* urediniospores by integrating an indirect immunofluorescence assay (IIFA) with direct propidium iodide (PI) staining. Monoclonal (Pp-mAb) and polyclonal (Pp-pAb) antibodies were produced, in mouse and rabbit respectively, in response to intact urediniospores. Spores were exposed to Pp-mAb or Pp-pAb and any binding was detected with a secondary antibody having a fluorescein isothiocyanate (FITC) label, followed by vital staining with PI. The spore-antibody complex was visualized using an epifluorescent microscope with an FITC/PI dual-emission filter. Under these conditions, both live and dead spores stained green but the nuclei of dead spores stained red, indicating the permeability of PI dye into the dead spores. This method can be greatly simplified into a two-step system by using a FITC-labeled Pp-mAb or Pp-pAb followed by incubation with PI to detect and quantify the relative amount of non-viable to viable *P. pachyrhizi* spores. This technique has been used as a research tool to confirm viability of urediniospores based on various treatments and has the potential to be used as an on-site system for forecasting soybean rust by monitoring the movement of airborne spores during the soybean-growing season.

Survey of diseases of agronomic switchgrass in Tennessee

A. L. VU (1), M. M. Dee (1), T. Russell (1), O. L. Fajolu (1), K. D. Gwinn (1), J. Zale (1), B. H. Ownley (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 100:S131

Switchgrass (*Panicum virgatum* L.) is a perennial warm-season (C4) native grass currently being investigated for use in biomass-based ethanol production in Tennessee. However, little is known about diseases that occur, or the impact of these diseases on the success of this crop. Reducing disease could significantly increase biomass yield and overall crop quality, particularly in the southeastern U.S. where large monocultures are being planted. The goal of this project was to identify fungal pathogens of switchgrass to gain an understanding of the role of disease in the overall efficiency and sustainability of switchgrass as a biofuel crop. Naturally infected 'Alamo' and 'Blackwell' switchgrass plants were collected from growers' fields in Vonore, TN in summer 2009 and agronomic research plots in Knoxville, TN in winter 2007 through spring 2008. Fungi were isolated from diseased plants and pathogenicity was confirmed with Koch's postulates in growth chamber studies. Several fungal pathogens were isolated also from seed. Pathogenic species of *Alternaria*, *Bipolaris*, *Curvularia*, and *Fusarium* have been identified; several of which have not been described previously on switchgrass, but are known to reduce quality and yield of other crops. Species identification was based on morphology of conidia and conidiogenous cells, colony characteristics, colony growth at various temperatures, and confirmed with internal transcribed spacer (ITS) sequences of ribosomal DNA.

USDA APHIS plant pest and biocontrol permitting and regulatory policy changes: Impacts on the stakeholder community

S. A. WAGER-PAGE' (1)
(1) USDA APHIS, Riverdale, MD, U.S.A.
Phytopathology 100:S131

USDA Animal and Plant Health Inspection Service (APHIS) published a proposed rule to amend 7CFR330, Plant Pest Regulations; Update of General Provisions, for public comment in October 2001. The update included provisions to implement permitting policy that the Plant Protection Act clarified and expanded. A final rule was not published for several reasons including the Agency's response to the events of September 11, 2001 and the Office of Inspector General (OIG) review recommending permitting policy changes. Plus, over 1,000 comments to the 2001 rule were received. Since 2002, the Pest Permitting Branch implemented numerous permitting policy changes based on the OIG review, Permitting Board of Advisor's recommendations and the development of the ePermits processing database. Standard permit conditions were developed and established for plant pest and regulated article movement types including interstate, importation, and continued curation. Amendments to permitting policy will be proposed for codification in 7CFR330 in the near future. APHIS continues to develop and upgrade ePermits. Eighty-five percent of pest permit applications are now received electronically, which has greatly improved processing times. APHIS continues to modify ePermits to further improve permit processing.

***Bacillus subtilis*, strain QST 713, Biofungicide II: Soil applications for disease control, yield improvement and quality enhancement**

P. J. WALGENBACH (1), D. Long (2), D. Sliva (3)
(1) AgraQuest, Inc., Davis, CA, U.S.A.; (2) AgraQuest, Inc., Demarest, GA, U.S.A.; (3) AgraQuest, Inc., Santa Maria, CA, U.S.A.
Phytopathology 100:S131

Bacillus subtilis, QST 713, is a soil borne strain of bacteria. 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 seemingly the result of a protective biofilm on the roots of plants, disease suppression from an array of lipopeptides and plant modulation. The practical implications of these new findings are discussed in regards to tomatoes, potatoes and cucurbits.

A new fungicide for control of Oomycete diseases of vine and vegetable crops

K. A. WALKER (1), J. S. Barnes (1), L. J. Newsom (2)
(1) BASF, Research Triangle Park, NC, U.S.A.; (2) BASF, Tifton, GA, U.S.A.
Phytopathology 100:S131

Zampro® is a new fungicide under development by BASF Corporation for control of Oomycete fungi including downy mildews and Phytophthora spp. Zampro is a premix containing two modes of action, dimethomorph and ametoctradin. Dimethomorph is a cell wall synthesis inhibitor classified as FRAC Group 40. Ametoctradin is a strong inhibitor of mitochondrial respiration classified as FRAC Group 45. Zampro is currently under trilateral review with Canadian (PMRA), US EPA and Australia (APVMA). Zampro was submitted for EPA registration for use on potato, grape, hop and vegetable crops. Results indicate Zampro exhibits excellent crop safety and disease control. Trial results from 2008 and 2009 and proposed directions for use will be presented. EPA registration is expected in 2012.

Multilocus phylogeny of *Ophiosphaerella* species causing spring dead spot of bermudagrass

N. WALKER (1), S. Marek (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S131

Ophiosphaerella herpotricha, *O. korrae*, and *O. narmari* are causal agents of spring dead spot on turf-type bermudagrass (*Cynodon* spp. and interspecific hybrids) in the transition zone of the United States. Growing as sterile mycelia in culture, *Ophiosphaerella* spp. cannot be identified morphologically and few DNA sequences are available for comparison. Forty *O. herpotricha*, twenty-seven *O. korrae* and five *O. narmari* isolates collected from bermudagrass from throughout the U.S. were selected for this study. Three *O. korrae* isolates from bluegrass were also included. DNA sequences of the nuclear ribosomal small subunit (SSU), internally transcribed spacer (ITS) region, and large subunit (LSU), a region of the translation elongation factor 1α (EF1α) gene, and the second largest subunit gene of RNA polymerase II (RPB2) from each isolate were analyzed. Group I introns of various sizes often were inserted in SSU sequences at up to four positions and corresponded with the species of the isolate. SSU introns often disrupted primer sites around the ITS region, precluding its use for identification. Multilocus phylogenies clustered *O. herpotricha* and *O. narmari* into well-resolved, monophyletic clades, while *O. korrae* isolates clustered more loosely due to greater sequence variation. EF1α and RPB2 sequences were more useful than rDNA sequences for identifying *Ophiosphaerella* isolates to species.

Death of epithelial cells in loblolly pine roots

C. H. WALKINSHAW (1)
(1) Columbus, GA, U.S.A.
Phytopathology 100:S131

Epithelial cells are widely distributed in biological systems where they line tissue such as resin ducts. These extremely thin cells are located adjacent to primary tracheids and large parenchyma cells. The objective of this study was to describe death of the epithelial cells in relation to root necrosis and enzyme hydrolysis. Histological and histochemical methods were used to process root tissues from field plantings. Tissues were fixed in buffered neutral formalin (ph 7.0) and stained with nine different schedules. Light microscopy results show there was a gradation in damage to the roots. Epithelial cells displayed

variation in necrosis and enzyme activity. Phenol (tannins) accumulated in the epithelial cells providing a good marker for predicting damage to loblolly roots. Further studies need to be conducted to better understand the nature of the tissue damage to the roots of loblolly pines.

Influence of foliar fungicide on components of grain yield in hybrid corn
M. W. WALLHEAD (1), L. Madden (1), P. Paul (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S132

Field experiments were conducted at two locations in Ohio (Wooster and South Charleston) to evaluate the effects of prothioconazole + trifloxystrobin, applied at different growth stages, on components of hybrid corn grain yield. Plots were planted with Pioneer hybrid 38A55 on 30 April at Wooster and 11 May at South Charleston, at approximately of 25,000 plants/ha at Wooster and 30,000 plants/ha at South Charleston. The experimental design was a randomized complete block, with treatments in a split-plot arrangement. Previous crop (corn or soybean) was the whole-plot and fungicide treatment (a non-treated check plus treatments applied at the R1, R2, or R3 crop growth stages) the sub-plot. All applications were made with a high-clearance sprayer at a rate of 356 ml of the fungicide per hectare and volume of 76 L/ha. At harvest, grain yield (YLD), test weight (TW), ear weight (EW), number of kernel rows per ear (KRE), and number of kernels per row (KR) were determined. Mean yield was significantly affected by location and cropping sequence, but not by fungicide treatment. Location also had a significant effect on TW and EW. However, other yield components were similar between the two locations and were not significantly affected by fungicide treatment nor cropping sequence. KRE and KR were fairly constant among treatments, with means ranging from 14 to 16 rows/ear and 28 to 38 kernels/row, respectively.

Do climate and outbreak frequency affect levels of foliar phytochemistry in different lodgepole pine (*Pinus contorta*) stands?
C. WALLIS (1), D. P. Huber (2), K. J. Lewis (2)
(1) USDA ARS, Parlier, CA, U.S.A.; (2) University of Northern British Columbia, Prince George, BC, CANADA
Phytopathology 100:S132

Lodgepole pine (*Pinus contorta* Douglas ex Louden) is a widely distributed tree in North American forests and is found in a variety of environments, each with different levels of disease activity. We quantified the levels of defense-associated metabolites (including soluble phenolics, lignin, and terpenes) in the foliage of 13 distinct lodgepole pine stands scattered throughout British Columbia to test the hypothesis that different climates would result in different levels of these compounds. Precipitation levels were positively correlated with soluble phenolic and terpenoid levels. Temperature was negatively associated with foliar lignin levels, implying that this compound affects cold hardiness. We also determined the frequency of past outbreaks of the foliar disease *Dothistroma septosporum* (Dorog.) Morelet) by using dendrochronological techniques and historical records in five of these stands, and then correlated outbreak frequency with the levels of secondary metabolites present in the foliage. The levels of lignin, soluble phenolics, and monoterpenes increased in direct relationship to frequency of disease outbreaks. Thus, disease outbreaks select for the production of defense-associated secondary metabolites in lodgepole pine foliage.

Races of *Puccinia striiformis* identified in the United States in 2009
A. WAN (1), X. Chen (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) USDA ARS, Pullman, WA, U.S.A.
Phytopathology 100:S132

Puccinia striiformis f. sp. *tritici* (PST) and *P. striiformis* f. sp. *hordei* (PSH) cause stripe rust on wheat and barley, respectively. To monitor virulence changes in the pathogen populations, stripe rust samples collected from 14 states were tested on 20 wheat and 12 barley differentials for identifying PST and PSH races, respectively. Six previously existing PSH races were detected, of which PSH-71 (virulent to Topper, Emir, Hiproly, Varunda, Abed Binder 12, Trumpf, Mazurka, Bigo, and Bancroft) was predominant. A total of 27 PST races were detected including new races PST-139 (virulent to Lemhi, Chinese 166, Heines VII, Paha, Druchamp, Produra, Yamhill, Stephens, Lee, Fielder, Tyee, Hyak, Yr8, Yr9, and Clement) and PST-140 (virulent to Lemhi, Heines VII, Moro, Produra, Stephens, Lee, Fielder, Tres, Express, Yr8, Yr9, Clement, and Compair). Races PST-139, PST-140, PST-114 (with all PST-140 virulences plus Yamhill), PST-116 (with all PST-114 virulences plus virulence to Paha), and PST-127 (with all PST-139 virulences, plus Moro, Express, and Compare) were predominant. The frequency of PST-127 increased from 1.5% in 2007 and 4.3% in 2008 in California and Washington to 9.1% in 2009 in California, Idaho, Oregon, and Washington. Twenty five of the 27 races were detected in the western U.S. while only five (PST-78, PST-80, PST-98, PST-100, and PST-102) were detected in the eastern U.S.

Trafficking of soybean cyst nematode secreted CLE proteins in plant cells
J. WANG (1), T. Hewezi (2), T. J. Baum (2), E. L. Davis (3), X. Wang (4), M. G. Mitchum (1)
(1) University of Missouri, Columbia, MO, U.S.A.; (2) Iowa State University, Ames, IA, U.S.A.; (3) North Carolina State University, Raleigh, NC, U.S.A.; (4) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S132

Soybean cyst nematodes (*Heterodera glycines*) produce secreted effector proteins that function as peptide mimics of plant CLAVATA3/ESR (CLE)-like peptides to promote parasitism. Similar to plant *CLEs*, nematode *CLEs* belong to gene families encoding small proteins with N-terminal signal peptides (SP), diverse variable domain (VD) sequences, and either a single or multiple, conserved C-terminal CLE domain(s) that are processed to release bioactive 12 or 13 amino acid (aa) CLE motif peptides. Previously, we determined that the nematode 12 aa CLE motif peptide is not sufficient for biological activity *in vivo*. Genetic and biochemical analysis confirmed the requirement of the VD and revealed a novel role in trafficking cytoplasmically-delivered CLEs to the apoplast in order to function as ligand mimics. VD deletion studies and yeast two-hybrid analysis are underway to identify essential motifs and/or interacting proteins required for protein trafficking. Subcellular fractionation will provide information about how these proteins traffic in plant cells. Together, these studies will help better understand the trafficking mechanism of nematode secreted CLE effector proteins in plants cells.

Numerical simulation of the long distance transports of wheat stripe rust pathogen in China
H. WANG (1), X. Yang (2), Z. Ma (1)
(1) Department of Plant Pathology, China Agricultural University, Beijing, PRC PEOPLES REP OF CHINA; (2) Department of Plant Pathology, Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S132

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an economically important disease of wheat worldwide. The objective of this study was to determine the connectivity between the source regions in China. Based on the meteorological data from 1997 to 2006, numerical simulation of the long distance transports of *P. striiformis* f. sp. *tritici* after oversummering and overwintering was conducted using HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) model. Total 25 and 27 source locations were selected from oversummering regions and from overwintering regions, respectively. The spores alive in the air for 120 hours were released at 00, 06, 12, and 18 UTC. The results indicated that in autumn the pathogen into northwestern China was mainly from southwestern China, that into Yunnan was mainly from Guizhou, and that into Guizhou was mainly from Yunnan. In spring, the spores into northwestern China were mainly from Sichuan and northern China, that into Guizhou were mainly from Yunnan and Sichuan, that into northern HuBei were mainly from northwestern, northern and southwestern China, and that into northeastern China were mainly from northwestern and northern China. Either in autumn or in spring, the spores into northern China were mainly from northwestern and southwestern China, and that into Sichuan were mainly from northwestern China, Yunnan and Guizhou. The inoculum sources in Xinjiang had little impact on wheat areas outside of Xinjiang.

Molecular diversity of *Sugarcane yellow leaf virus* in China
M. Wang (1), D. Xu (1), G. ZHOU (2)
(1) Laboratory of Plant Virology, South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA; (2) South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S132

Sugarcane yellow leaf virus (SCYLV, genus *Polerovirus*, family *Luteoviride*) has become widespread in many sugarcane growing regions in China during the past decade, and a new aphid vector, *Ceratovacuna lanigera*, was recently found to transmit the virus in the field. To investigate the molecular diversity of this virus, an RT-PCR-RFLP method was developed for genotype discrimination based on viral CP and MP coding sequence, from which the RT-PCR amplicons (1326 bp) displayed polymorphism between the recognized four genotypes ie. BRA, PER, REU and CUB genotype, after the endonuclease *Bam*H I, *Hin*d III and *Xho* I digestion. 514 samples were collected from 53 field locations in southern China, from the year 2007 to 2009. It was revealed that 81 (15.8%) samples were SCYLV positive in the RT-PCR detection, and 59 of them were identified as BAR genotype, whereas other 22 displayed unexpected RFLP patterns in the RFLP analysis. Furthermore, the amplicons from these 22 samples were cloned and sequenced. Sequence comparison indicated that 10 of them shared high nucleotide similarities with the recognized genotypes, 4, 4, and 2 samples grouped into BAR, PER, and CUB genotypes, respectively, and the remaining 12 samples, based on their nuclotide identity data, could be divided into two

groups, each of which might represent a new SCYLV genotype for they had distinct differences (less than 96.0% nt identies) with the recognized gentotypes.

Comparative analysis of the RcsC sensor kinase from *Erwinia amylovora* and other enterobacteria

D. WANG (1), Y. Zhao (1)
(1) University of Illinois, Urbana, IL, U.S.A.
Phytopathology 100:S133

RcsC is a hybrid sensor kinase which contains a sensor domain, a histidine kinase domain and a phosphoreceiver domain. We have previously demonstrated that, though the *Erwinia amylovora rcsC* mutant produces more amylovoran than the wild type strain *in vitro*, it is avirulent on the host plants. In this study, we further characterized the RcsC and its homologs from various enterobacteria. Our results showed that overexpression of *Erwinia* RcsC suppressed amylovoran production in different amylovoran over-producing strains, indicating net phosphatase activity of RcsC. Complementation studies showed that RcsC homologs from *Pantoea stewartii* and *Yersinia pestis*, but not those from *E. coli* and *Salmonella enterica*, partially restored virulence of the *Erwinia rcsC* mutant on gala apple shoots. However, all RcsC homologs could not rescue amylovoran production phenotype of the *Erwinia rcsC* mutant. In addition, a chimeric construct containing the sensor domain of *Erwinia* RcsC and the output domain of *E. coli* RcsC restored virulence of the *Erwinia rcsC* mutant, but not amylovoran production; whereas a chimeric construct containing the sensor domain of *E. coli* RcsC and the output domain of *Erwinia* RcsC rescued amylovoran production phenotype of the *Erwinia rcsC* mutant, but not virulence. These results suggest that the sensor domain of RcsC might be essential for regulating bacterial virulence, whereas the output domain of RcsC might be responsible for regulating amylovoran production.

Evaluation of foliar response of cucumber to *Phytophthora capsici* and inoculation techniques

X. WANG (1), M. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S133

Phytophthora blight, caused by *Phytophthora capsici* Leonian, has become a limiting factor for cucumber (*Cucumis sativus*) growers in Michigan. Pre- and post-emergence damping-off are very common symptoms of *P. capsici* infection on cucurbit crops. Cucumber seedlings were used to investigate the infection of *P. capsici* on the foliage of cucurbits under controlled laboratory conditions. Four *P. capsici* isolates (OP97, SP98, 12889 and 13351) were employed to determine the disease response on the foliage of susceptible cucumber cultivar 'Vlaspik' by using three inoculation techniques including mycelial plug, droplet and spray. Disease symptoms appeared on the cotyledons one day after inoculation. Significant differences ($P \leq 0.05$) among AUDPC values calculated for the isolates and inoculation techniques were observed respectively. No significant interaction was found between isolates and inoculation techniques. Isolate 12889 was most virulent when 20 ml of zoospore suspensions (1×10^6 per ml) were used and applied via foliar droplet or spray. The foliar-droplet inoculation technique was quantitative, effective and consistent in evaluating the disease response on cucumber seedlings compared to the other two inoculation methods. Therefore, isolate 12889 and the foliar droplet technique will be used to evaluate Phytophthora foliar blight of cucurbits.

First report of new strains of *Puccinia striiformis* f. sp. *tritici* pathogenic to Zhong 4 (*Tititrigia*) in China

B. WANG (1), X. Hu (1), Q. Li (1), G. Li (1), Z. Kang (1)
(1) Plant Protection College and Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A&F University, Yangling, Shaanxi, PRC PEOPLES REP OF CHINA
Phytopathology 100:S133

Tititrigia Zhong 4 has good agricultural properties and high resistances, and has been used as an important germplasm resource in wheat breeding all over the world. It was assigned as one of 17 differential hosts in race identification of *Puccinia striiformis* f. sp. *Tritici* (PST) since 1983 due to its immunity to all races. Until now, only Zhong 4 is resistant to all races of PST in China. It has been taken as an efficient resistant material for wheat stripe rust by many breeders. In 2007, 6 wheat leaf samples (named as T1 to T6) among 196 samples from Taibai in Shaanxi province were found to be pathogenic to Zhong 4 with reaction type 4. The reaction type of T2, T4, and T6 on differential hosts was very similar with race Su11-11 except for those on Zhong 4. The severity of three isolates on Zhong 4 was 25%. The incidence was 66.7%, 75.0%, and 60.0%, respectively. Thus, T2, T4, and T6 should be new strains. The pathogenicity of the three strains to seeding of wheat cultivars or germplasm materials was tested in field from 2008 to 2009. Results showed that three strains were toxic to most of cultivating varieties or germplasm materials (near 84.2% among 247). The reaction type, incidence, and severity of the three strains were lower than those of race CYR31, CYR32, CYR33, and Su11-4 on most of varieties or germplasm materials. The parasitic fitness of them was apparently lower than that of current epidemic races.

Use of a strip-till cover crop system to manipulate above and below ground organisms in cucurbit plantings

K. WANG (1), C. R. Hooks (2), S. P. Marahatta (1)
(1) University of Hawaii, Honolulu, HI, U.S.A.; (2) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S133

A field trial was conducted in 2008 and 2009 to evaluate the potential of using sunn hemp (SH), *Crotalaria juncea*, and marigold (MG), *Tagetes patula* in a strip till cover cropping (STCC) system to suppress crop pests and increase the number of free-living soil organisms involved in nutrient cycling. Cucumber (*Cucumis sativus*) and winter gourd (*Benincasa hispida*) were planted as cash crops in 2008 and 2009, respectively. In 2008, SH increased the abundance of free-living nematodes (including bacterivores, fungivores, and omnivores) and soil microarthopods (detritivores) compared to the monoculture bareground (BG) treatment and suppressed the total abundance of plant-parasitic nematodes by the end of the cucumber cropping cycle. Thrips and whitefly numbers on cucumber were reduced significantly in SH compared to BG treatment only in 2008. However, due to more severe melon fly (*Bactrocera cucurbitae*) colonization in SH plots, SH had lower cucumber yield than the BG. Similar results were obtained in 2009, except that SH did not reduce foliar insect pests. Winter gourd seedlings were initially infected by *Microphomina phaseolina*, but more seedlings in the SH plots survived the infection than those in the BG. Final yield of winter gourd was also significantly higher in the SH than BG plots. In conclusion, STCC with SH managed above and below ground beneficial organisms and pests efficiently and resulted in increased in crop yield in the second year of the STCC system.

The striatin ortholog of *Colletotrichum graminicola* plays a role in mycelial growth, conidiation, and virulence to maize

C. WANG (1), W. Shim (1), B. D. Shaw (1)
(1) Dept. Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S133

Colletotrichum graminicola is a foliar pathogen of maize causing anthracnose and stalk rot. In filamentous fungi, striatin orthologs have been implicated in multiple developmental processes including sexual development and pathogenicity, and localize to the endoplasmic reticulum and the nuclear envelope. However, little is known about its role in *C. graminicola*, a heterothallic, hemi-biotrophic pathogen of maize. We generated *C. graminicola str1* deletion mutants using split-marker recombination. Mutants grew slower on culture media, and lost the clockwise spiral growth of wild type on PDA. Interestingly, the mutants gained a counter-clockwise spiral growth pattern on V8 medium. The *str1* mutants produced shorter falcate conidia and fewer, less developed acervuli on nutrient medium and autoclaved maize leaf, resulting in reduced falcate conidium production. Oval conidium production was low, which may reduce the secondary infection in stalk colonization. The *str1* mutants still produced appressoria and successfully penetrated leaves, but were delayed in foliar lesion development. In stalk rot assays, mutants produced reduced lesions at the primary infection sites. Our study indicated that the *str1* deletion mutants were defective in several propagation-associated developmental events and were less virulent to maize.

Detection of *Phytophthora* species in retail nurseries and urban forest environments in northern Nevada

S. WANG (1), S. Garneni (1)
(1) Nevada Department of Agriculture, Sparks, NV, U.S.A.
Phytopathology 100:S133

To survey for *Phytophthora ramorum*, *P. kirnoviae*, and other *Phytophthora* species in retail nurseries and urban forest environments, a total of 385 symptomatic plant samples were collected from 140 host species or varieties in 27 nurseries and 28 urban environments. To isolate *Phytophthora* species, fresh leaf tissue or phloem and xylem tissue was placed on the selective medium PARP. Isolates of *Phytophthora* were then transferred to corn meal agar and V8 juice agar for morphological identification. Molecular identification was employed by amplifying and sequencing an rDNA region containing partial 18S ribosomal RNA gene, ITS1, 5.8S ribosomal RNA gene, ITS2 and 28S ribosomal RNA gene. Of the total of 12 isolates obtained, 8 were from urban maple trees showing a bleeding canker symptom, and 4 were from plants showing leaf blight in retail nurseries. *P. cactorum* was predominantly associated with maple bleeding canker (88%), whereas *P. citricola* was found only from one maple tree. In nurseries, *P. cactorum* was found from Fraser's photinia, *P. citricola* from Red Robin cinquefoil, and *P.*

citrophthora from both Canadale Gold euonymus and Vicary Golden privet. This survey suggests that *P. cactorum* is the major cause of chronic decline and death of maples in urban environments of northern Nevada and that nursery stock carrying various *Phytophthora* species is a direct pathway of introducing non-native pathogens into the urban forest environments.

Field evaluations of *Simplicillium lanosoniveum* as a biological control agent for *Phakopsora pachyrhizi*
N. A. WARD (1), R. W. Schneider (1), C. L. Robertson (1)
(1) Louisiana State University Agricultural Center, Baton Rouge, LA, U.S.A.
Phytopathology 100:S134

Simplicillium lanosoniveum is an inhabitant of soybean rust sori where it parasitizes urediniospores and affects sorus development. Microscopic observations indicated that the filamentous fungus penetrated and colonized urediniospores within 5 days of inoculation. To further evaluate the impact of this mycoparasitic fungus, we conducted field tests in 2009 near Baton Rouge, LA to evaluate *S. lanosoniveum* as a biological control agent. Treatments included foliar applications of conidia of *S. lanosoniveum* at various plant growth stages and rust severities. Leaf samples were collected weekly from late vegetative stages through senescence and were rated for disease severity and total number of sori. Next, we subjected total leaf genomic DNA, including all associated microorganisms, to qPCR analyses in order to quantify *S. lanosoniveum* and *P. pachyrhizi*. We found that *S. lanosoniveum* colonized and survived on leaf surfaces for 6 weeks after the earliest inoculation. This led to delayed disease development and reduction in numbers of sori. We conclude that *S. lanosoniveum* has mycoparasitic properties that should be exploited for biological control of soybean rust and possibly other rust diseases. This field of investigation incorporates ecological principles into the study of phyllosphere microbiology, which may lead to alternative means of disease control.

Development of ELISA and qPCR for *Squash vein yellowing virus* detection
C. WEBSTER (1), W. Li (2), C. Kousik (3), S. Adkins (1)
(1) USDA ARS, Ft. Pierce, FL, U.S.A.; (2) UF, Lake Alfred, FL, U.S.A.; (3) USDA-ARS, Charleston, SC, U.S.A.
Phytopathology 100:S134

Watermelon vine decline caused by *Squash vein yellowing virus* (SqVYV) is a new and emerging disease that has caused severe losses to Florida watermelon growers in recent years. First identified in 2005, SqVYV is widely distributed in southwest and west-central Florida and has recently been found infecting several cucurbit weeds, often without inducing symptoms. Although late stage symptoms of the disease are basically diagnostic for the presence of SqVYV, earlier symptoms are not as obvious and may be confused with other causes. Thus, continued development of simple and reliable diagnostic tests for early monitoring of SqVYV in watermelon and cucurbit weeds remains important as accurate identification is the first step in management. After several unsuccessful attempts to produce specific antisera from virion preparations, peptides of the SqVYV coat protein (CP) were synthesized and used to immunize rabbits. The resulting polyclonal antisera were tested in ELISA and found to react with SqVYV but to none of the other cucurbit-infecting viruses common in Florida. A real-time PCR assay is being developed that targets the CP gene of SqVYV. Initial tests have shown the PCR assay to be sensitive and specific for SqVYV. These newly developed ELISA and real-time PCR methods for SqVYV detection are being compared to existing methods of detection including: conventional RT-PCR, tissue blots and indicator hosts on both greenhouse grown and field collected samples.

Identification of *Groundnut ringspot virus* in tomato in south Florida
C. WEBSTER (1), L. Horsman (2), G. Frantz (2), C. Mellinger (2), S. Adkins (1)
(1) USDA ARS, Ft. Pierce, FL, U.S.A.; (2) GCC, Jupiter, FL, U.S.A.
Phytopathology 100:S134

Fresh market tomatoes are widely grown in Florida and are subject to infection by several viruses. *Tomato spotted wilt virus* (TSWV) is common in north Florida tomato production areas and is occasionally found in south Florida. Tomato plants with typical tospovirus symptoms (including necrotic flecking, ring patterns, irregular chlorotic areas, and deformation of leaves; and necrotic lesions on the epidermis of petioles and stems) were seen in November 2009 and February 2010 in south Florida, after being observed sporadically for about a decade. Several thrips species were observed in these recent symptomatic tomatoes including *Frankliniella bispinosa*, *F. occidentalis*, *F. schultzei* and *Thrips palmi*. No TSWV was detected by ELISA or RT-PCR tests in samples collected at either time but infection by another tospovirus was indicated by ELISA and RT-PCR tests using broad spectrum tospovirus antiserum and degenerate tospovirus primers, respectively. Subsequent ELISA tests did not detect other tospoviruses known to occur in the U.S. However, ELISA tests using an antiserum that reacts with both *Groundnut ringspot virus* (GRSV) and *Tomato chlorotic spot virus* were positive. A 697 nt fragment of the nucleocapsid (N) gene sequence amplified by RT-PCR was >95% identical to GRSV N gene sequences in Genbank confirming the presence of GRSV. Additional characterization of the Florida GRSV isolate from tomato is ongoing.

Rapid micro-dilution broth assay for evaluating in vitro fungicide resistance in *Botrytis cinerea*
D. E. WEDGE (1), K. J. Curry (2), B. Kreiser (2), A. Curry (2), M. Abril (3), B. J. Smith (4)
(1) USDA ARS NPURU, University, MS, U.S.A.; (2) Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS, U.S.A.; (3) Department of Biological Sciences, University of Southern Mississippi, Baton Rouge, LA, U.S.A.; (4) USDA ARS Southern Horticultural Laboratory, Poplarville, MS, U.S.A.
Phytopathology 100:S134

Recently strawberry growers in southeastern Louisiana reported a failure of fungicide spray programs to control Botrytis fruit rot. Since *Botrytis cinerea* has become resistant to several commonly used fungicides classes we suspected chemical insensitivity. A 96-well micro-dilution broth assay using a 3-point dose response protocol developed for fungicide discovery was used to provide growers with a rapid assessment of the fungicide sensitivity profiles of 13 *Botrytis* isolates; 12 obtained from the strawberry farms and a control isolate obtained from blueberry. Fungicide sensitivity profiles were established for each of 13 isolates against 11 fungicides based on mean percent growth inhibition. We identified 3 phenotypes in the sensitivity profiles to benzimidazole and dicarboximide fungicides: benzimidazole and dicarboximide resistant, benzimidazole resistant and dicarboximide sensitive, and those with benzimidazole and dicarboximide intermediate resistance. Codon at position 198 in the β-tubulin gene confirmed benomyl resistance of 10 of 11 strawberry isolates. Traditional fungicide sensitivity assays are tedious, time consuming, and often large pathogen populations are sampled that do not provide a rapid answer for extension personnel or growers. Our assay provides a method to rapidly obtain resistance and sensitivity information and allow growers to incorporate important chemical disease management strategy during the disease cycle when they need it.

***Brassica juncea* seed meal amendment induces long-term suppressiveness to *Pythium abappressorium* under enclosed and open soil incubation conditions**
M. WEERAKOON (1), A. Izzo (2), M. Mazzola (3)
(1) Washington State University, Wenatchee, WA, U.S.A.; (2) Elon University, Elon, NC, U.S.A.; (3) USDA ARS, Wenatchee, WA, U.S.A.
Phytopathology 100:S134

Pythium spp. contribute to development of apple replant disease. *B. juncea* seed meal (SM) soil amendment can effectively suppress *Pythium* via generation of biologically active allyl isothiocyanate (AITC). AITC is evacuated from soils within 48 h after SM application, yet preliminary evidence indicates that long-term control of this pathogen may be attained. Greenhouse trials were conducted to assess the capacity of *B. juncea* SM to suppress P. abappressorium in AITC evacuated soils. SM was incorporated into soil at a rate of 0.3% (wt\wt); one system was maintained under enclosed conditions and the other open to the atmosphere for two days. Soils were then incubated in slightly covered containers to maintain moisture conditions. Soils were incubated for 2, 4 and 8 weeks and then infested with *P. abappressorium*. Regardless of the time of pathogen introduction after *B. juncea* SM amendment, disease suppression was consistently observed under both enclosed and open initial incubation conditions. However, disease suppression was significantly greater in soils incubated under enclosed conditions for the initial two days relative to soils incubated under open conditions during the same period. Evacuation of AITC from these soils prior to infestation with *P. abappressorium* suggests that disease suppression does not operate completely via chemical means. Disease suppression was associated with distinct changes in the resident fungal community prior to pathogen introduction.

Validation of the accuracy of single-kernel near-infrared technology to sort winter wheat kernels based on scab and deoxynivalenol levels
S. WEGULO (1), K. Peiris (2), P. Baenziger (1), F. Dowell (2)
(1) University of Nebraska, Lincoln, NE, U.S.A.; (2) USDA-ARS, Manhattan, KS, U.S.A.
Phytopathology 100:S134

Fusarium head blight (scab) of wheat caused by *Fusarium graminearum* lowers yield and grain quality. *F. graminearum* also produces the mycotoxin deoxynivalenol (DON) which contaminates grain. For purposes of quality assurance, DON concentration usually is determined in grain or the products made from it. One of the methods commonly used to measure DON is gas chromatography (GC). Although GC is accurate, it is time consuming, costly, and destructive since grain must be ground to flour before DON can be measured. For purposes such as breeding for resistance to FHB, it may be

sufficient early in the breeding program to know only whether a given line accumulates low or high DON. A rapid, inexpensive, and non-destructive method is needed for such purposes. This study validated the accuracy of two single-kernel near-infrared (SKNIR) systems to sort winter wheat kernels based on scab and DON levels. Both SKNIR systems accurately discriminated between wheat kernels with low and high DON levels.

An investigation into mixed infections by potato purple top and potato witches'-broom phytoplasmas in tomato

W. WEI (1), W. Wu (1), I. Lee (1), R. E. Davis (1), R. A. Owens (1), D. L. Nuss (2), Y. Zhao (1)
(1) Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD, U.S.A.; (2) Center for Biosystems Research, University of Maryland Biotechnology Institute, Rockville, MD, U.S.A.
Phytopathology 100:S135

In nature, plants are often infected by two or more pathogens simultaneously. Mixed infections may induce "atypical" symptoms that make precise visual diagnosis difficult. The impact of mixed infections on the host can be severe, especially when the co-infecting agents interact synergistically. Potato purple top (PPT) and potato witches'-broom (PWB) phytoplasmas are newly characterized pathogens that cause serious diseases in potato and other vegetable crops. While PWB phytoplasma is a member of subgroup 16SrVI-A, several PPT-associated phytoplasma strains belonging to subgroups 16SrVI-A, 16SrXII-A, 16SrIII-M, and 16SrIII-N have been identified. The wide distribution of their insect vectors make mixed infections inevitable. In the present work, we developed 16S rRNA gene sequence-based molecular markers and a sensitive diagnostic tool to study mixed infections by a 16SrVI-A PPT phytoplasma strain and a PWB phytoplasma strain in tomato plants. The distribution and relative abundance of the two co-infecting phytoplasmas were monitored over a 60-day post-infection time course and for five passages in plants. Our results revealed that i) the two competing phytoplasmas differ in fitness level for tomato; ii) interactions between the two phytoplasmas induce new symptoms unseen in infection by either phytoplasma alone; and iii) the severity of the symptoms is correlated with the relative abundance of the two phytoplasmas.

Grafting as a disease management tool for fusarium wilt of heirloom tomatoes in Arkansas

K. D. WELCH (1), J. C. Correll (1), J. C. Gavin (2)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) University of Arkansas Cooperative Extension Service, Warren, AR, U.S.A.
Phytopathology 100:S135

The cultivation of grafted vegetables began in Korea and Japan in the early 20th century and has been adopted by many countries to control soilborne pathogens. Grafting of tomatoes was adopted in the 1960's. *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), causes Fusarium wilt of tomato, and is an economically important disease in the commercial tomato production area of Arkansas. There are 3 known races of *Fol* and all 3 have been found in Arkansas. While there are a number of fresh-market tomato cultivars grown in Arkansas that have resistance to all 3 races, a number of heirloom tomatoes do not have adequate resistance. The heirloom cultivar Bradley, developed as a pink fresh-market tomato, is commonly grown in Arkansas and is valued for its color, flavor, and stable price. Bradley is only resistant to race 1 of *Fol*. The objective of this study was to determine if Bradley could be grafted onto a rootstock resistant to races 1, 2, and 3 and provide control of *Fol*. The cultivar Crista, resistant to all 3 races, was used as the rootstock. Ungrafted Bradley, Bradley grafted onto Bradley, and ungrafted Crista served as controls. Disease incidence and severity was evaluated under inoculation conditions in a greenhouse test and in commercial fields naturally infested with *Fol*. If disease control and adequate yields can be obtained, grafting Bradley onto a resistant rootstock may provide a means of growing an heirloom susceptible tomato in fields naturally infested with *Fol*.

Development of a national standard for virus certification of ornamental and fruit tree nursery stock

R. Welliver (1), M. Hansen (2), N. OSTERBAUER (3)
(1) Pennsylvania Dept. of Agriculture, Harrisburg, PA, U.S.A.; (2) Michigan Dept. of Agriculture, St. Joseph, MI, U.S.A.; (3) Oregon Dept. of Agric, Salem, OR, U.S.A.
Phytopathology 100:S135

Since the 1960s, U.S. nurseries have traded virus-certified *Malus*, *Pyrus*, *Prunus*, *Chaenomeles* and *Cydonia* nursery stock interstate and internationally. A hindrance towards trade has been the lack of a harmonized national standard for virus certification of such stock. Instead of reviewing a national standard, importers must assess several unique state regulations designed to meet their import requirements. Adoption of RSPM No. 35 by the North American Plant Protection Organization has also obliged movement towards a national standard. A subcommittee of the Fruit Tree Clean Plant Network, including members of federal and state agencies, academia, and industry met to develop such a standard in November 2009. A draft was developed that contained several key elements including: 1) Harmonization of the language used in state regulations for virus certification programs; 2) Creating a two-tiered approach towards virus certification; and, 3) Setting minimum standards for each tier of certification. Efforts were made to harmonize the list of viruses of concern, taking regional differences into account. Language describing the use of a systems approach to maintain virus-certified status was included. A pilot study will be conducted in Pennsylvania, Michigan, and Oregon to assess the efficacy of the proposed national standard. It is anticipated the presence of the standard will facilitate trade interstate and with other countries throughout the world.

Mutational analysis of the putative *pipo* of *Soybean mosaic virus* with emphasis on symptom expression and virus accumulation

R. Wen (1), B. He (1), M. R. HAJIMORAD (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.
Phytopathology 100:S135

The function(s) of *pipo*, a newly discovered open reading frame (ORF) embedded in the P3 cistron of potyviruses, is largely unknown. The putative *pipo* of *Soybean mosaic virus* (SMV) is 225 nucleotides long, encoding for 75 amino acids, and has a GA6 motif at its 5′-end. We recently showed that disruption of SMV PIPO protein, without substitution in polyprotein ORF, did not abolish virus replication, but restricted the resultant mutants to small foci of infected cells within the inoculated leaves. Furthermore, extensive mutagenesis of the conserved GA6 motif also generated two movement-defective *pipo*-mutants. We are now studying the putative *pipo* of SMV by mutational analysis to find out whether it influences the expression of symptom severity and enhancement of virus accumulation. To achieve these goals, the differential interactions of three SMV strains with soybean cv. Williams82 are being exploited. SMV-N induces severe symptoms and accumulates to a high level in systemically infected leaves, while SMV-G7 and SMV-G7d accumulate at relatively lower levels and provoke mild symptoms. Interestingly, SMV-N PIPO protein differs from those of SMV-G7 and SMV-G7d by three amino acid substitutions whereas PIPO protein of SMV-G7 is differentiated from that of SMV-G7d by a single residue. Analyses of reciprocal exchanges of the unique amino acid residues of PIPO proteins of these strains, individually or in combination without substitutions in polyprotein ORF, are underway.

Impact of Zebra Complex disease on the development of potato plants from seed-borne infection of '*Candidatus* Liberibacter solanacearum'

A. WEN (1), X. Wang (1), J. S. Pasche (1), N. C. Gudmestad (1)
(1) North Dakota State University, Fargo, ND, U.S.A.
Phytopathology 100:S135

An emerging disease of potatoes known as 'Zebra Chip' or 'Zebra Complex'(ZC), is putatively caused by the fastidious, phloem-limited bacterium '*Candidatus* Liberibacter solanacearum' (Lso), vectored by potato psyllid, *Bactericera cockerelli*. Although ZC is spreading, the role of seed-borne ZC in dissemination of the disease is not known. Seed tubers from the same certified seed lot, with and without ZC symptoms, were grown in the greenhouse to assess the impact of the disease on germination, progeny tuber production and chip discoloration. The presence of Lso was tested in seed tubers, foliage and progeny tubers. Lso was detected in both ZC-symptomatic and asymptomatic seed, however, Lso concentration in asymptomatic seed was substantially lower. Significant differences in emergence between ZC-symptomatic and asymptomatic seed were observed. Plants emerged from non-ZC seed appeared healthy with 99% emergence while plants emerged from ZC-seed displayed typical ZC symptoms with 42% emergence. No plants grown from asymptomatic seed were Lso positive, whereas 29/64 plants derived from ZC-seed were Lso positive. A high percentage (99%) of progeny tubers from non-ZC seed was asymptomatic, whereas among plants from ZC-seed, only 44% produced progeny, 34% of which displayed ZC symptoms. None of the progeny tubers from asymptomatic seed were Lso positive, however, 11/43 of progeny tubers from ZC-seed were positive. Chip discoloration in progeny tubers of ZC-seed generally was more severe than those from non-ZC seed.

Alternative fumigants for management of root-knot nematode on carrots

B. B. WESTERDAHL (1), J. S. Gerik (2)
(1) Nematology Dept., University of California, Davis, CA, U.S.A.; (2) USDA, ARS, San Joaquin Valley Agricultural Center, Parlier, CA, U.S.A.
Phytopathology 100:S135

Two field trials were conducted to evaluate the effectiveness of iodomethane (MI) and chloropicrin (CP) for management of root-knot nematode, *Meloidogyne javanica*, on carrots. Treatments in both trials were CP at 337 kg/ha, a 50/50 formulation of MI/CP at 374 kg/ha, a 33/67 formulation of MI/CP at 122, 187, and 288 kg/ha, and a water treated control. Treatments

were applied pre-plant, under tarp, via drip irrigation, and each trial consisted of 5 replications in a randomized complete block design. In the first trial, compared to the control, all MI/CP treatments increased yield of marketable carrots based on both number of carrots and weight of carrots (95%). MI/CP at 187 kg/ha (95%) had lower levels of root-knot nematode present at harvest than the control. In the second trial, compared to the control, MI/CP at 187 and 288 kg/ha increased yield of marketable carrots based on both number of carrots and weight of carrots (95%). MI/CP at 288 kg/ha (90%) had lower levels of root-knot nematode present at harvest than the control.

Population density development of *Heterodera schachtii* under susceptible, resistant and tolerant sugar beet cultivars

A. WESTPHAL (1), M. Daub (2)
(1) Julius Kühn-Institut. Federal Research Centre for Cultivated Plants, Toppheideweg 88, D- 48161 Münster, GERMANY; (2) Julius Kühn-Institut, Federal Research Centre for Cutlvated Plants, Dürener Strasse 71, D-50189 Elsdorf, GERMANY
Phytopathology 100:S136

Heterodera schachtii is a severe problem in sugar beet. Cultural management of *H. schachtii* may be facilitated by the use of resistant and tolerant sugar beet cultivars. To examine effectiveness of such cultivars, we monitored population dynamics of *H. schachtii* in 30 cm-diameter plots, infested with 550 *H. schachtii* eggs/100 g of soil at different depths: 0-60 cm, 0-30 cm, or 30-60 cm; non-inoculated layers and the control were filled with non-infested soil. Plots were planted to a susceptible, resistant or tolerant sugar beet cultivar. Root penetration rates of seedlings by juveniles of *H. schachtii* were similar for the cultivars. Early canopy diameter was larger in non-inoculated than in entire column-inoculated plots. Final population densities were cultivar-specific as expected due to their host suitability and independent of initial inoculation depth. White sugar yields were highest in non-infested soil, next highest in the deep-infested soil, and lowest in the all-layers-infested soil. In two trials in 1 m^2 naturally infested with *H. schachtii* at 0-60 cm depths, populations were suppressed with fosthiazate at 30 cm-depth layers reciprocal to the artificially inoculated ones of the first trial. In one of these trials, yields were highest under nematicide treatment and lowest under non-treated. In both types of trials, deep-occurring populations of *H. schachtii* reached sugar beet seedlings and caused damage. Deep-occurring populations of *H. schachtii* must not be ignored in sustainable sugar beet production.

Sampling for pod rot of peanut

T. A. WHEELER (1), J. E. Woodward (2), S. A. Russell (3), M. G. Cattaneo (4)
(1) Texas AgriLife Research, Lubbock, TX, U.S.A.; (2) Texas AgriLife Extension, Lubbock, TX, U.S.A.; (3) Texas AgriLife Extension, Brownfield, TX, U.S.A.; (4) Texas AgriLife Extension, Seminole, TX, U.S.A.
Phytopathology 100:S136

Two peanut fields with a history of pod rot were sampled weekly at 101 randomly chosen locations. Each sample location covered 0.5 m of row. The objective was to determine a sampling intensity which would adequately estimate pod rot for three treatment thresholds. Low, medium, and high treatment thresholds were chosen at ≥1%, ≥2.5%, and ≥5.5% pod rot. Pythium pod rot was the primary disease at both sites. From the sampled locations, 5, 10, 15, 20, 25, 35, and 50 sample points were selected at random from the data sets, with 10 simulations for each sampling intensity. In general, when a threshold number was close to the average pod rot, there were more wrong threshold decisions than when the average pod rot was far away from a threshold number. There were more wrong decisions for the high threshold, compared with the moderate and low thresholds. It was more difficult to be accurate at the higher percentage of pod rot than the lower disease levels when sampling up to 50 locations (of the 101 sampled points). For the low and moderate thresholds, 15 sampling points was as likely to be accurate as higher sampling intensity, and more likely to be accurate in terms of being over or under threshold than with lower sampling intensity. Crop consultants must determine how intensive to sample to identify disease problems timely, but must also maintain profitability while scouting fields, by minimizing time spent in a field. This project is aimed at assisting consultants.

Root susceptibility and inoculum production from roots of eastern oak species to *Phytophthora ramorum*

T. L. WIDMER (1), N. Shishkoff (2), S. Dodge (2)
(1) USDA ARS FDWSRU, Frederick, MD, U.S.A.; (2) FDWSRU/ ARS/USDA, Frederick, MD, U.S.A.
Phytopathology 100:S136

Little is known about root susceptibility of eastern tree species to *Phytophthora ramorum*. In this study, we examined root susceptibility and inoculum production from roots. Roots of sprouted acorns for several eastern oak species were exposed to zoospore suspensions of 1, 10, 100, or 1000 zoospores per ml at 20°C. After 24 h, roots were removed, rinsed in water, planted in pots and placed in the greenhouse. After 4 weeks, the roots were surface sterilized and plated on PARPH+V8 medium. A root was recorded as positive if *P. ramorum* was observed on the medium. Infection of oak radicles occurred at a concentration as low as 1 zoospore per ml. Differences were observed among the species tested. To test inoculum production, the roots of oak seedlings were inoculated with sporangia, washed after 24 hr and transplanted into 2 × 2 inch pots containing Turface®. Periodically, 20-25 ml samples of runoff were collected from each pot and plated on PARPH; the resulting colonies were counted. Counts from oaks were compared to a positive control, *Viburnum tinus*, using regression analysis. Root segments were plated to calculate percent colonization. After 16 days, inoculum production from oak seedlings was variable and lower than *V. tinus*, as was colonization of roots. After 35-days, results were similar. This study shows that sprouted oak acorns are very susceptible to *P. ramorum* and may be important epidemiologically under natural environmental conditions.

Efficacy of new fungicides for control of powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) of grapes

W. F. WILCOX (1), D. G. Riegel (1)
(1) Cornell University, NY State Agr Expt Sta, Geneva, NY, U.S.A.
Phytopathology 100:S136

Materials representing two new classes of fungicides, metrafenone (220 g/ha) and fluopyram, provided superior control of powdery mildew relative to current products. Fluopyram was more effective at 180 vs 86 g/ha; the lower rate mixed with 88 g/ha tebuconazole was equivalent to the higher rate solo. Among newly-available DMI fungicides, tetraconazole (44 g/ha) and flutriafol (73-91 g/ha) were roughly equivalent and often modestly superior to traditional DMIs; flutriafol was significantly more effective at 225 g/ha than at the lower rates. Difenoconazole (128 g/ha) was significantly more effective than all other DMIs; perhaps causally, EC50 values of individual *E. necator* isolates averaged 29-fold lower (i.e., more active) than those for myclobutanil. Tank mixing difenoconazole with cyprodinil (368 g/ha) did not improve control, and cyprodinil solo gave modest to no control, depending on pressure. Fluopicolide, BAS 651 (ametoctradin + dimethomorph), and mandipropamid (146 g/ha) at 14-day spray intervals all provided excellent control of downy mildew under high disease pressure. Fluopicolide was numerically but not statistically more efficacious at 140 vs. 105 g/ha; tank-mixing with copper hydroxide did not improve its performance. BAS 651 was equivalent at 422 vs 537 g/ha and at 10- vs 14-day intervals. Cyazofamid (80 g/ha) solo was slightly less effective than the above, but was equivalent when tank-mixed with a phosphite product.

Global gene expression analysis of *Pseudomonas syringae* during epiphytic and endophytic growth

J. L. WILLIAMS (1), X. Yu (2), R. Scott (3), S. P. Lund (2), A. R. Records (4), G. A. Beattie (2), S. E. Lindow (3), D. C. Gross (1), D. Nettleton (2)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) Iowa State University, Ames, IA, U.S.A.; (3) University of California- Berkeley, Berkeley, CA, U.S.A.; (4) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S136

Pseudomonas syringae is an important plant pathogen with a prominent epiphytic phase that serves as inoculum for subsequent infection. To obtain a comprehensive understanding of the genetic networks contributing to saprophytic and pathogenic growth, we are performing whole genome transcriptional profiling of *P. syringae* pv. *syringae* B728a, a pathogen of bean, using an ORF-based microarray. Gene expression of the wild-type strain and *ahlR*, *aefR*, *gacS*, *salA*, *retS*, *rpoE*, *rpoN*, *rpoS*, and *hrpL* mutants are being assessed on leaf surfaces and in the leaf apoplast as well as in culture in a basal medium and under conditions of low iron, nitrogen and water availability and high oxidative stress. Total RNA was collected from cells recovered from epiphytic and endophytic sites from at least 600 and 60 leaves, respectively, to obtain yields sufficient for analysis. Preliminary results have indicated that the B728a cells experience distinct environments during these distinct growth phases based on differences in gene expression. For example, genes involved in flagellar motility and type VI secretion were expressed more in epiphytic sites, whereas genes involved in syringomycin production, levan synthesis, uptake of quaternary ammonium compounds, GABA degradation and tolerance to water stress and nitrogen limitation were expressed more in endophytic sites. The importance of the targeted regulatory networks to the distinct growth phases of B728a is currently being evaluated.

Phenotyping the components of resistance as a bottleneck to breed rice varieties with suitable resistance to sheath blight

L. WILLOCQUET (1), M. Noel (1), N. Magculia (1), J. Lore (2), A. Srinivasachary (1), S. Savary (1)
(1) IRRI, Los Banos, PHILIPPINES; (2) PAU, Ludhiana, INDIA
Phytopathology 100:S136

Sheath blight (ShB) caused by *Rhizoctonia solani*, is a rice disease causing important yield losses, particularly under intensive production systems. Host

plant resistance to ShB can represent a very efficient, pro-poor, and environment-friendly way to manage this disease. No high resistance against ShB has been deployed in Asia. Phenotyping is a key component in breeding programs aiming at improving host plant resistance, particularly when dealing with quantitative resistance, as in the case of ShB. We present here a framework that allows to measure host plant resistance from physiological resistance and from disease escape. A phenotyping method under controlled conditions was developed to measure the components of physiological resistance in terms of number of lesions, disease spread, and lesion expansion. A phenotyping procedure in microfields was also developed to measure the combined effects of physiological resistance and disease escape on disease intensification and spread. The results obtained from a series of tests performed on a range of rice genotypes, and using both approaches (controlled conditions phenotyping, microfields), allow assessing the relative contributions of physiological resistance and disease escape to the overall resistance of rice to ShB.

Multi-state evaluation of integrated management strategies for Fusarium head blight and deoxynivalenol in small grain

K. T. WILLYERD (1), C. Bradley (2), A. Grybauskas (3), D. Hershman (4), L. Madden (1), M. McMullen (5), L. Osborne (6), L. Sweets (7), P. Paul (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.; (2) University of Illinois, Urbana, IL, U.S.A.; (3) University of Maryland, College Park, MD, U.S.A.; (4) University of Kentucky, Princeton, KY, U.S.A.; (5) University of Minnesota, St. Paul, MN, U.S.A.; (6) South Dakota State University, Brookings, SD, U.S.A.; (7) University of Missouri, Columbia, MO, U.S.A.
Phytopathology 100:S137

Field experiments were conducted in multiple states between 2006 and 2009 to determine the magnitude of Fusarium Head Blight (FHB) and deoxynivalenol (DON) reduction achieved by integrating multiple management strategies, relative to a single strategy. Experiments used a randomized complete block design with a split-plot arrangement of fungicide treatment and cultivars of wheat, barley or durum, depending on region. Some trials incorporated previous crop for a split-split-plot arrangement. A combination fungicide of prothioconazole and tebuconazole was applied at anthesis to cultivars with varying levels of FHB susceptibility. Disease index was assessed at soft dough and DON concentration was determined following harvest. In general, fungicide, host resistance and cultivation of small grain following a non-host crop reduced FHB and DON. However, the magnitude and significance of individual and combined effects of these management strategies varied among experiments. Compared to a susceptible check, the moderately resistant cultivar reduced index by –4 to 98% and DON by 3 to 96%. Compared to untreated checks for each cultivar, fungicide alone reduced index by 15 to 85% and DON by -157 to 74%. By combining resistance and fungicide, index and DON were reduced by 26 to 99% and 23 to 82%, respectively. A three-tier management approach of crop rotation, resistance and fungicide reduced index and DON by 27 to 99% and 61 to 92%, respectively.

Effectiveness of early-season fungicide programs for the control of *Sclerotinia homoeocarpa*, the causal agent of dollar spot

C. WILSON (1), P. Koch (1), J. Kerns (1)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.
Phytopathology 100:S137

Dollar spot, caused by *Sclerotinia homoeocarpa*, is the most important turfgrass disease in the United States with respect to fungicide expenditures. Single early-season fungicide applications delay dollar spot symptom development, but do not provide season long control of the disease. 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 C.C. in Wisconsin. Conventional applications started June 1 and were applied on 14-day intervals using full label rates of propiconazole and chlorothalonil. Early-season treatments were applied May 1, followed up with applications of a tank mixture of propiconazole and chlorothalonil at either ¾ rates every 21 days or full label rates applied on 28-day intervals. Treatments were arranged in a randomized complete block design with four replications with individual plots measuring 2.8 m^2. Disease severity was rated visually by counting individual dollar spot foci every two weeks. The 21-day early-season program suppressed dollar spot development, but not to acceptable levels (<5% disease severity). The 28-day early-season program provided an excellent suppression that was comparable to the conventional program. One fungicide application could be eliminated by using a 28-day early season program rather than a 14-day conventional program, reducing fungicide expenditures and environmental inputs.

Effects of temperature on growth of *Sclerotinia homoeocarpa*

C. WILSON (1), J. Kerns (1), D. Smith (2)
(1) University of Wisconsin-Madison, Madison, WI, U.S.A.; (2) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S137

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 WI and 6 isolates from OK were grown on native soils and sand. WI isolates were grown with and without creeping bentgrass (*Agrostis stolonifera*) debris and incubated at temperatures of 11, 14, 17, 20, 23, 26, 29, 31 and 34°C. OK isolates were grown with creeping bentgrass 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. Growth for all isolates was most rapid between 17 and 26°C. WI isolates grew best on native silt loam with bentgrass debris. Growth was significantly reduced and highly variable at temperatures below 15°C. These data suggest that *S. homoeocarpa* is strongly saprophytic, and that higher temperatures (17–26°C) are conducive to growth. To assess pathogen aggressiveness, 3 WI isolates and 6 OK isolates were inoculated on live creeping bentgrass incubated at 14, 20, 26 or 34°C. Disease severity was assessed every 24 hours. Four days post-inoculation, disease was most severe at 14 and 20°C for all isolates, with average severity as high as 25%. These initial data suggest that *S. homoeocarpa* infects creeping bentgrass between 14 and 26°C.

Exploring the role of nitroalkane dioxygenases in *Magnaporthe oryzae* morphogenesis and infection

R. WILSON (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, U.S.A.
Phytopathology 100:S137

Rice blast, mediated by *Magnaporthe oryzae*, is the most destructive disease of rice. Foliar infection is mediated by a specialized infection structure called the appressorium. Generation of cellular turgor in the mature appresorium forces a penetration peg through the rice leaf cuticle and allows the fungus access to the plant interior. Once inside the host cell, the fungus forms an intimate association with the plant cell and is able to suppress or neutralize the host defense response and grow unimpeded for the first 72 hrs of infection. We are interested in understanding the molecular and cellular processes that underlie this important interaction. Nitroalkanes are produced by some plant species in response to pathogen attack, and can also result from ROS damage to cellular alkanes such as fatty acids. We have identified five genes encoding nitroalkane dioxygenases that could neutralize the harmful affects of nitroalkanes produced during plant infection. Homologous gene replacement of two of these genes affects sporulation and growth of the fungus. Moreover, susceptibility to nitroalkanes is increased in one of these mutant strains during growth on cysteine, compared to wild type. Together, this work suggests that nitroalkane dioxygenases are important for the normal development of the fungus and might protect *M. oryzae* against harmful products of the plant defense system. Characterization of the remaining nitroalkane dioxygenases, and their role in virulence, will be discussed.

Interactions of the endophyte *Acremonium zeae* and *Aspergillus flavus* in maize hybrids in the field

G. L. WINDHAM (1), W. P. Williams (1)
(1) USDA ARS, Mississippi State, MS, U.S.A.
Phytopathology 100:S137

The maize endophyte *Acremonium zeae* has recently been shown to produce pyrrocidines which are toxic to a number of fungi commonly found in maize kernels including *Aspergillus flavus*. Field studies were conducted to determine the effect of *A. zeae* on aflatoxin accumulation and *A. flavus* kernel infection of two maize hybrids. *Acremonium* inoculation methods included injecting conidia under the husks of ears, spraying conidia on silks, and inoculating stalks just below developing ears with infested toothpicks. *Aspergillus flavus* inoculation methods included the side-needle and spray techniques. In 2007 in three experiments where *A. zeae* was inoculated using three different methods, aflatoxin accumulation was significantly higher in *A. flavus*-resistant hybrid plants inoculated with both *A. zeae* and *A. flavus* than in plants inoculated with *A. flavus* alone. *Aspergillus flavus* kernel infection was also significantly higher in plants inoculated with both fungi using the side-needle technique compared to plants inoculated with *A. flavus* alone. In 2008, aflatoxin accumulation was significantly higher in the resistant hybrid inoculated with both fungi using the side-needle technique compared to plants inoculated with *A. flavus* alone. Although *A. zeae* has been reported as a protective endophyte, we demonstrated it can act synergistically with *A. flavus* to produce higher levels of *A. flavus* kernel infection and aflatoxin accumulation in a resistant maize hybrid.

Emergence and establishment of *Cucurbit yellow stunting disorder virus* in California and Arizona poses a threat to desert melon production

W. M. WINTERMANTEL (1), R. L. Gilbertson (2), E. T. Natwick (3), J. K. Brown (4)
(1) USDA ARS, Salinas, CA, U.S.A.; (2) Department of Plant Pathology, University of California, Davis, CA, U.S.A.; (3) University of California Desert Research and Extension Center, Holtville, CA, U.S.A.; (4) Department of Plant Sciences, University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S138

Cucurbit yellow stunting disorder virus (CYSDV; genus *Crinivirus*, family *Closteroviridae*) was identified in the large melon production region of the American Desert Southwest (CA, AZ, SON) in fall 2006, and affected most fall melon crops in the region. CYSDV is transmitted efficiently by the sweet potato whitefly (*Bemisia tabaci* biotype B). Whitefly populations accumulate gradually during the spring melon season, but reach high levels during the fall melon season. Analysis of weeds and crops in and adjacent to infected fields, along with subsequent laboratory studies, demonstrated that the host range of CYSDV included not only cucurbits as previously believed, but also several crop and weed plants native to the region. Many of these hosts do not exhibit symptoms, but can be sources for virus transmission to crops. Over a period of 3 years, all fields in Imperial County, CA and fields in central Arizona were monitored for CYSDV during both spring and fall production seasons, and whiteflies from fields were tested for CYSDV. During this period, CYSDV incidence in the fall crop was nearly 100%, resulting in a dramatic reduction in fall production and yields. In contrast, incidence in spring melons was initially low and limited to a small number of fields in 2007, but increased to 63% of fields by spring 2009, indicating establishment in native vegetation and an increasing threat to the spring crop. Southwest production accounts for 80% of the U.S. cantaloupe crop and 96% of the U.S. honeydew melon crop.

Response by *Aspergillus flavus* to a sublethal atmosphere of ozone

C. P. WOLOSHUK (1), L. Zhang (1), B. N. Reese (1), G. A. Payne (2)
(1) Purdue University, Department of Botany and Plant Pathology, West Lafayette, IN, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S138

Ozone is a powerful oxidant with numerous beneficial applications. When fungi are exposed to a sublethal atmosphere of ozone, surface growth and development are inhibited. To better understand the molecular responses to ozone, liquid cultures of *Aspergillus flavus* were grown for 3 days in an ozone atmosphere. Fungal growth below the surface of the medium was not affected, but aerial growth was suppressed. In contrast, cultures exposed to air produced abundant aerial mycelia and conidia. When the ozone-treated cultures were shifted to an air environment, aerial hyphae were visible after 4 h and conidia were visible after 24 h. Total RNA was isolated from cultures 0, 4, 12 and 24 h after removal from ozone and from cultures grown in an air environment. The RNA was hybridized to microarrays that contain probes representing 14,163 *A. flavus* genes. Expression profiles indicated that transcription of hydrophobins and conidiation genes were significantly reduced in the ozone-treated cultures. Among the few genes significantly up-regulated in ozone-treated cultures was *CAT5*, one of five putative catalase genes in *A. flavus*. Within 4 h after shifting the ozone-treated cultures to an air environment, *CAT5* expression decreased to control levels. Furthermore, when cultures grown in air were shifted to the ozone environment, *CAT5* expression increased 3-fold after 4 h. Based on these results, we hypothesize that *CAT5* has a role in protection against ozone.

Ontogenic resistance to powdery mildew in hop cones: Implications for disease management

J. L. WOODS (1), M. E. Nelson (2), G. G. Grove (2), D. H. Gent (3)
(1) USDA ARS, Corvallis, OR, U.S.A.; (2) Washington State University, Prosser, WA, U.S.A.; (3) USDA-ARS/Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 100:S138

Podosphaera macularis, causal agent of hop powdery mildew, can cause substantial losses in crop yield and quality. Ontogenic resistance has been described in leaves, although this resistance has not been examined in detail in cones. Greenhouse produced cone tissues were inoculated on a time course to assess their susceptibility to powdery mildew in different developmental stages. Field-based fungicide programs also were evaluated to determine the impact of omitting late-season fungicide applications on cone yield, bittering acids, and quality factors. In greenhouse assays, flowers were highly susceptible to powdery mildew, but susceptibility of bracts and bracteoles decreased linearly with increasing cone maturity. In fungicide trials conducted under high disease pressure, there was a tendency for later season fungicide applications (up to 24 August) to improve cone yield, alpha acid content, and quality. The incidence of diseased cones was correlated with alpha acid content (r = –0.62; P = 0.04), cone color (r = –0.62; P = 0.01), and aroma quality (r = –0.77; P = 0.01). Under low disease pressure, however, cone yield, alpha acid content, and quality were similar if fungicide applications were made through 27 July. Cone color was negatively affected in treatments that ended before this date. Further characterization of ontogenic resistance in cones may enable control measures to be targeted to critical periods of cone susceptibility and potentially reduce unnecessary fungicide applications.

Effects of irrigation and crop rotation on Verticillium wilt of cotton in Texas

J. E. WOODWARD (1), T. A. Wheeler (2), J. P. Bordovsky (2)
(1) Texas AgriLife Extension Service, Lubbock, TX, U.S.A.; (2) Texas AgriLife Research, Lubbock, TX, U.S.A.
Phytopathology 100:S138

Verticillium wilt, caused by the soilborne fungus *Vertcillium dahliae*, is an increasingly important disease of cotton (*Gossypium hirsutum* L.) on the Southern High Plains of Texas. Disease incidence is correlated with soil populations of the fungus and management options are currently limited to the use of partially resistant cultivars. The impact of other cultural practices on disease development, or *V. dahliae* is unknown. Field trials were conducted to determine the influence of three irrigation levels (Base, Base + 50% and Base – 50%) on disease development under four rotation schemes (continuous cotton, and three rotations containing sorghum (*Sorghum bicolor* L.)). Disease incidence increased from 0.9 to 14.5% and 4.0 to 19.3% for the low and high ET treatments in 2008 and 2009, respectfully. Populations of *V. dahliae* increased from 2.7 to 50.7 cfu/cc soil over a three year period where cotton was planted, whereas, populations remained similar where sorghum was planted. These studies indicate that lower irrigation levels and rotation with a non-host can negatively impact *V. dahliae* and result in lower disease incidence. Additional studies are required to optimize the use of such practices to ensure sustainable cotton production in fields infested with *V. dahliae*.

Evaluation of the edge factor in epidemiology of zebra chip disease in potato fields

F. WORKNEH (1), D. C. Henne (1), A. C. Childers (1), C. M. Rush (1)
(1) Texas AgriLife Research, Bushland, TX, U.S.A.
Phytopathology 100:S138

Zebra chip (ZC), caused by the fastidious bacterium '*Candidatus* Liberbacter solanacearum', is an emerging disease of potatoes that affects all market classes. Initially observed in Mexico in the mid 1990s, the disease now has been identified in several potato producing regions of the U.S. including Texas. The pathogen is vectored by the potato psyllid, *Bactericera cockerelli*, and affected plants exhibit a variety of foliar symptoms, and unacceptable discoloration of fried potato products (chips and french fries). In 2009, studies were conducted in three fields in the Texas Panhandle to determine whether there was a greater incidence of ZC on the edges of fields than within fields. Plots of 20 m x 10 m were established around the edges of the fields (50 ha each), approximately160 m apart (n = 15 to 18). The same number of plots also were established 100 m inward (corresponding to individual plots on the edges), and symptomatic plants in all plots were counted. In all three fields, ZC incidence was significantly greater ($P < 0.05$) on the edges than in the infield plots, and some directional effects were evident. In a separate study, temporal progression of ZC on the edges was monitored in two fields in which new symptoms were recorded weekly. In both fields, ZC incidence peaked 2 weeks after the first detection and declined steeply thereafter. The findings suggest that greater emphasis in psyllid management should be directed towards the edges of the fields for better results.

Evaluation of southern highbush blueberry cultivar and propagation methods for stem blight mortality during the first year of growth in Florida

A. F. WRIGHT (1), P. F. Harmon (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S138

Stem blight is caused by fungi in Botryosphaeriaceae; infection results in substantial southern highbush blueberry (SHB) mortality during the first two years of growth. Cultivar and propagation method effects on Botryosphaeria (Bot) disease incidence and severity were evaluated. Three commercial sites were selected and are located in Alachua, DeSoto, and Polk, Co. FL. Plot installation was a RCBD and consisted of four cultivars each propagated from softwood cuttings (sw) and tissue culture (tc). The cultivars Emerald, Premadonna, and Snowchaser were planted at all locations. Star was planted at Alachua, and Jewel was planted at the DeSoto and Polk, Co. sites. Plots were sampled periodically for stem blight, and data were analyzed with ANOVA. For the Alachua Co. site very few plants died and the model was not significant $p > 0.45$. At the Desoto and Polk Co. sites, cultivar and propagation had a significant effect on plant mortality $p < 0.05$. Plants propagated from tc survived more frequently and had less stem blight than plants propagated from sw. Cultivar differences in Bot susceptibility observed

in this study are similar to reports of relative cultivar susceptibility in production fields. New cultivar development presents a long-term potential management option for stem blight. Additionally, industry-wide propagation practices need to be reviewed and improved with the input of the industry and results of further research.

AV2 protein encoded by Tomato yellow leaf curl China virus is a RNA silencing suppressor

J. Wu (1), J. Zhang (1), X. ZHOU (1)
(1) Hangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S139

GFP green fluorescence in the leaves of 16c transgenic *N. benthamiana* plants co-infiltrated with the A. tumefaciens harboring *GFP* gene and the A. tumefaciens harboring Tomato yellow leaf curl China virus (TYLCCNV) isolate Y10 *AV2* gene could be observed and a narrow red ring around the edge of infiltrated patch could also be found at 6 days post inoculation (dpi), which indicated that TYLCCNV Y10 AV2 protein can suppress local RNA silencing of *GFP* gene, but can not interfere with the spread of RNA silencing signal of *GFP* gene by cell-to-cell. In addition, *GFP* green fluorescence could be observed in the systemic leaves of 16c transgenic *N. benthamiana* plants co-infiltrated with GFP and *AV2* genes at 30 dpi, which suggested that TYLCCNV Y10 AV2 protein can inhibit systemic RNA silencing of *GFP* gene. Moreover, TYLCCNV Y10 AV2 protein could interfere with the spread of systemic RNA silencing signal of GFP gene by agrobacterium-mediated infiltration assay. The above results indicated TYLCCNV Y10 AV2 protein is a RNA silencing suppressor. TYLCCNV Y10 AV2 protein is a pathogenicity determinant in the PVX heterogenous system. Northern blot analysis indicated that the concentration of PVX RNA was higher in the *N. benthamiana* infiltrated with PVX-AV2 than that in the *N. benthamiana* infiltrated with PVX. It could be speculated that the higher concentration of PVX in the *N. benthamiana* infiltrated with PVX-AV2 was due to the AV2 protein suppressing the antivirus RNA silencing of the plants.

Effects of sampling methods on the assessment of populations of *Xanthomonas hortorum* pv. *carotae* on carrot plants and on harvested carrot seeds

B. M. WU (1)
(1) Oregon State University, Madras, OR, U.S.A.
Phytopathology 100:S139

To optimize sampling techniques for assessing population levels of *Xanthomonas hortorum* pv. *carotae* (*Xhc*) in carrot seed crops, populations of *Xhc* on carrot plant parts and on harvested carrot seeds were estimated by using different sampling methods. The influence of sample methods, including the number of samples, sample size (the dry weight and the number of plants per tissue sample, or the number of seeds per seed sample), and number of subsamples, on the estimation of mean bacterial population size (Log CFU) per gram dry tissue or dry seed and on the variability among sampling units was investigated. In general, bacterial populations were highly variable among sampling units. The variation in population size of *Xhc* was greater (0.53 to 3.59 Log CFU) among plants than among subsamples (0.007 to 0.48 Log CFU) suggesting that a good sample method should collect samples from as many plants as possible, and increasing number of subsamples or the number of medium plates for enumeration cannot improve the estimate of population size as much as increasing the number of plants sampled. The sample size (the number of seeds per sample) is important in estimation of the bacterial population on harvested seeds, and a large sample size is especially important to accurately estimate the bacterial population for seed lots with low infestation levels. The population sizes among sample units sometimes, but not consistently, followed a lognormal distribution.

Determining the development of practical fungicide resistance in cucurbit powdery mildew of pumpkin

C. A. WYENANDT (1)
(1) Rutgers University, Bridgeton, NJ, U.S.A.
Phytopathology 100:S139

In 2006 and 2007, nine fungicides were evaluated to determine if practical fungicide resistance could be identified in cucurbit powdery mildew (*Podosphaera xanthii*) following five different season-long fungicide programs using different modes-of-action and rotations. The nine fungicides evaluated included: sulfur (FRAC code M2), chlorothalonil + myclobutanil (M5 + 3), famoxadone + cymoxanil (11 + 27), myclobutanil (3), azoxystrobin (11), pyraclostrobin + boscalid (11 + 7), pyraclostrobin (11), quinoxyfen (13), chlorothalonil (M5) and a control (water only). Based on percentage of leaf surface with symptoms of powdery mildew, a FRAC code 11 resistance cucurbit powdery mildew population developed where a FRAC code 11 fungicide had not be applied directly season-long, or where a FRAC code 11 fungicide had been applied weekly or in rotation with another fungicide chemistry. Resistance did not develop where a FRAC code 3 fungicide had been applied season-long, or in rotation, or where no FRAC code 3 fungicide had been applied season-long. Control was improved with a FRAC code 7 + 11 fungicide, suggesting that control of the cucurbit powdery mildew population in this study was achieved with the FRAC code 7 fungicide. In both years, of the nine fungicides evaluated during this study, end of season cucurbit powdery mildew control was best with a FRAC code 3, 11 + 7, or 13 fungicide.

Sensitive and cost-effective immunocapture RT-PCR for routinely viral detection in large number of plant samples

J. Q. XIA (1), K. Ling (2)
(1) AC Diagnostics, Inc., Fayetteville, AR, U.S.A.; (2) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, U.S.A.
Phytopathology 100:S139

It has long been a challenging task to develop timely and accurate diagnostic tests for diseases caused by viruses or virus-like agents in plants in agriculture production. Current ELISA detection methods may not provide the sensitivity needed for samples with low viral concentrations. Conventional RT-PCR, which requires relatively pure nucleic acid samples, is costly and time-consuming. By combining two widely used virus detection methods, ELISA and RT-PCR, Immunocapture RT-PCR was developed for practical detection of plant viruses. The immunocapture sample preparation allows RT-PCR to be performed in a 96-well format equivalent to that of a regular ELISA test. The entire RT-PCR assay, viral immunocapturing from samples, captured viral RNA amplification and amplicon detection, can be conducted in a single PCR reaction tube within a relatively short time. This novel technology makes it possible to routinely detect plant viruses in large number of samples by RT-PCR. This sensitive and cost-effective immunocapture RT-PCR assay has huge potential applications in plant viral disease diagnosis.

Development of commercial products based on immunocapture RT-PCR technology

J. XIA (1), C. Feng (2), K. Ling (3)
(1) AC Diagnostics, Inc., Fayetteville, AR, U.S.A.; (2) Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR, U.S.A.; (3) USDA-ARS, U.S. Vegetable Laboratory, Charleston, SC, U.S.A.
Phytopathology 100:S139

A sensitive, reliable and user-friendly commercial product has been developed based on immunocapture RT-PCR technology. This diagnostic kit includes all assay components and is ready to use. Pre-coated PCR tubes for capturing virus particles which can be directly amplified by RT-PCR allows entire assay to be performed in a single PCR tube. Sample is simply ground in a PCR sample extract buffer and added to PCR plate/tubes pre-coated with antibody. After incubation and washing, the captured target viral RNA is ready to be amplified by RT-PCR. Robust and consistent result can be obtained every time running a RT-PCR by using this product because all PCR inhibitors are eliminated through viral immunocapture. This immunocapture RT-PCR product provide one of the most sensitive diagnostic tools for an effective detection of plant viruses in seeds, stock materials or other plant tissues of economically important vegetables, fruits, ornamentals and field crops.

Cuticle plays an important role in basal as well as induced defense against bacterial and fungal pathogens

Y. XIA (1), Q. Gao (1), K. Yu (1), A. Kachroo (1), P. Kachroo (1)
(1) University of Kentucky, Lexington, KY, U.S.A.
Phytopathology 100:S139

Systemic acquired resistance (SAR) is a phenomenon in plants that confers protective immunity in the distal tissues towards secondary infections by related or unrelated pathogens. SAR involves the generation of mobile signal (s) at the site of primary infection, which then translocates to, and activates defense responses in the distal tissues. Although several signals have been implicated to play a role in SAR, the signaling events leading to activation of SAR still remains unclear. Recently, we showed an intact cuticle is required for decoding of the mobile signal in the distal tissues. Genetic mutations leading to abnormal cuticle or physical damage of cuticle on the distal leaves compromised SAR. The requirement for intact cuticle was only relevant within the time frame of mobile signal generation and translocation to the distal tissues. Since most mutations affecting cuticle development also impair fatty acid (FA) and/or lipid biosynthesis, we studied a role for these in SAR. Our results show impaired biosynthesis of FAs or lipids do not contribute to SAR. we have uncovered several mutations that specifically alter cuticle without influencing FA or lipid biosynthesis and they were impaired in SAR. Besides SAR, most mutants with abnormal cuticle showed enhanced susceptibility to necrotrophic fungal pathogens and this phenotype did not correlate with cuticular permeability. The studies demonstrate an important role for cuticle in induced as well as basal defense responses.

Gene regulation during asexual development in the oomycete *Phytophthora infestans*

Q. XIANG (1), H. Judelson (1)
(1) Dept. of Plant Pathology and Microbiology, University of California, Riverside, CA, U.S.A.
Phytopathology 100:S140

Phytophthora infestans, the causal agent of potato and tomato late blight, undergoes a serial of distinct morphological differentiations during asexual reproduction. Our study focuses on identifying the genes involved in differentiation, and the transcription factors and cognate promoter binding sites that are responsible for the expression of these genes. Microarray studies revealed that thousands of genes were up-regulated in different stages of asexual reproduction. These genes encode proteins involved in flagellar function, vesicle transport, protein posttranslational modification, signaling and other activities that may be important in asexual development. Dissections of the promoters of several genes identified transcription factor binding sites required for their developmental regulation. One of the binding sites is CCGTTG, which is significantly enriched in promoters specifically active in sporulation. As CCGTTG is known to bind MYB transcription factors from animals and plants, we characterized the MYB transcription factor families from four sequenced oomycete genomes. Several groups of MYB proteins were predicted, with some resembling those found in plants and animals and others having oomycete-specific configurations of their DNA binding domains. Many of these myb genes are differentially expressed during asexual development based on RT-PCR. Gene silencing and over-expression experiments are being conducted to determine how MYB proteins regulate the spore cycle.

Characterization of *Sclerotiium rolfsii* isolates affecting vegetables and row crops in the southern U.S.

C. XIE (1), G. Vallad (1)
(1) University of Florida - Gulf Coast Res & Ed Ctr, Wimauma, FL, U.S.A.
Phytopathology 100:S140

Southern blight (caused by *Sclerotium rolfsii* Sacc.) is a serious fungal disease affecting diverse crops grown around the world especially in tropical and subtropical regions. While mostly a problem in the production of peanut in the southern U.S., the disease is becoming more problematic in vegetable production with the phase out of methyl bromide and the adoption of organic and other low-input production strategies. An initial 38 isolates from peanut, pepper, tomato, pumpkin, cantaloupe, watermelon, and a *Ruellia* sp. were partially characterized for cultural morphology and mycelial compatibility. An initial characterization of fourteen isolates found variation in the number and size of sclerotia produced on PDA. Most peanut isolates produced less than 250 sclerotia per plate, while most isolates from other hosts produced more. Sclerotia size ranged from from 0.60mm to 0.80mm in diameter for four isolates from pepper, cantaloupe, and watermelon; whereas, 7 of 9 peanut isolates ranged from 1.14mm to 1.49mm, with the remaining two isolates measuring 0.76mm and 0.93mm in diameter. The 38 isolates were assigned to 14 MCGs. No common MCGs were observed among peanut and vegetable isolates, with the exception of a single peanut isolate. Initial results suggest vegetable isolates are distinct from peanut isolates, but further characterization of additional isolates is in progress. Isolates will also be tested for differences in virulence on peanut, pepper, and tomato.

Visualization of *Clavibacter michiganensis* subsp. *michiganensis* infection of tomato seedlings using a bioluminescent strain

X. XU (1), G. Rajashekara (1), S. A. Miller (1)
(1) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S140

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is a Gram-positive bacterium that causes severe economic losses in commercial tomato production worldwide. The disease is transmitted from infected seed to seedlings, and mechanically from plant to plant. To study *Cmm* movement from tomato seed and seedlings, healthy tomato seeds were inoculated with a virulent, bioluminescent *Cmm* strain (BL-Cmm17) by vacuum infiltration. The transmission and localization of BL-Cmm17 from seed to seedling was monitored under controlled conditions using an *in vivo* imaging system (IVIS) and culturing. Our results indicated that *Cmm* aggregated on hypocotyls and cotyledons at the early stage of germination. *Cmm* transmission was also investigated in tomato plants inoculated by cotyledon clipping with BL-Cmm17. The bacterial infection process was visualized every 5 days. The bacteria multiplied quickly in the petiole remaining after clipping the cotyledon and reached a titre of 10^8 cfu/g fresh tissue 5 days post inoculation. Translocation of bacteria to stem tissue was also observed with a titre of 10^6 to 10^7 cfu/g. The first true leaf was infected without symptoms 10 days post inoculation. Leaf wilting was observed 15–20 days post inoculation and the bacterial titre in stem reached 10^9 cfu/g.

A screening strategy of fungal biocontrol agents towards Verticillium wilt of cotton

L. Xu (1), J. GUO (1)
(1) Nanjing, PRC PEOPLES REP OF CHINA
Phytopathology 100:S140

Verticillium wilt caused by Verticillium dahliae Kleb. is one of the most destructive diseases in cotton. A screening strategy was developed to assess the potential biocontrol agents (BCAs) of this disease. 373 fungal isolates were obtained from the endorhiza, rhizosphere, and bulk soil of cotton plants. 105 of them produced obvious inhibition zones against V. dahliae were selected as the antagonists towards this pathogen. An assessment system was established to evaluate these 105 antagonists for their biocontrol potential and plant growth-promoting potential. Their biocontrol potential was assessed according to their in vitro antagonistic activity against V. dahliae and activities of plant cell wall degrading enzymes including protease, cellulase, and chitinase. Their plant growth-promoting potential was assessed according to their in vitro activities of solubilizing phosphate and fixing nitrogen. 33 antagonists received at least 3 points of the total assessed value of biocontrol potential and plant growth-promoting potential; they were tested for biocontrol efficacy and growth-promoting effect on cotton in greenhouse. 12 of them achieved positive biocontrol efficacy of 8.58–69.78% in greenhouse. Their biocontrol efficacy is positively correlated with their assessed biocontrol potential, the correlation coefficient is 0.926.

Biological characterization and complete genomic sequence of *Apium virus Y* infecting celery

D. Xu (1), H. LIU (2), S. T. Koike (3), F. Li (1), R. Li (1)
(1) USDA-ARS, Beltsville, MD, U.S.A.; (2) USDA ARS, Salinas, CA, U.S.A.; (3) University of California Cooperative Extension, Salinas, CA, U.S.A.
Phytopathology 100:S140

Apium virus Y (ApVY) isolated from celery plants with ring spot and line pattern symptoms from a commercial field in California was characterized in this study. The experimental host range of the virus included 14 plant species in the families *Apiaceae*, *Chenopodiaceae* and *Solanaceae*, and almost all infected plant species showed foliar chlorosis and distortion or severe stunting and systemic chlorosis. ApVY was transmitted to all 10 host species in the *Apiaceae* by green peach aphids (*Myzus persicae*) and induced symptoms on the inoculated plants. The virus reacted with the potyvirus group antibody and *Celery mosaic virus* (CeMV) antiserum. The complete genomic sequence of ApVY was determined to be 9917 nucleotides in length, excluding the 3' poly(A) tail, and it comprises a large open reading frame encoding a polyprotein of 3184 amino acid residues. Its genomic organization is typical of potyviruses, and contains conserved motifs found in the genus *Potyvirus*. Comparisons with available sequences of other potyviruses indicate that ApVY shares 26.1–52.9% identities with species of the existing genera and unassigned viruses in the *Potyviridae* at the polyprotein sequence level. Extensive phylogenetic analysis based on 3'-partial sequences containing NIb and CP genes confirms that ApVY is more closely related to CeMV and is a distinct species of the genus *Potyvirus*.

Multiple infection of *Sugarcane streak mosaic virus* in a single sugarcane plant and complete genomic sequences of two SCSMV genotypes

D. XU (1), G. Zhou (2), F. Li (1), R. Li (1)
(1) USDA ARS, Beltsville, MD, U.S.A.; (2) South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S140

At least four genetic variants of *Sugarcane streak mosaic virus* (SCSMV) were identified from a single sugarcane accession (Co 6304) in the germplasm collections of South China Agricultural University at Guangdong, China. Complete genomic sequences of two Co6304 variants were determined to be 9782 nucleotides (nt). Comparison of the full-length sequences among these variants and a Pakistan isolate shows that they share identities of only 87.5–88.2% at the nt sequence level, but 97.9–98.2% at the polyprotein sequence level. Most mutations are point and silent, resulting in highly conserved polyprotein sequences. Similar results are also observed from all individual genes except the coat protein (CP) gene. Comparison of the CP gene sequences of four Co6304 variants and 62 isolates available in GenBank reveals a wide range of divergences not only at the nt sequence level (0–18.1%) but also at the amino acid sequence level (0–14.3%). Phylogenetic analysis based the CP gene sequences shows that these isolates are grouped into four distinct clusters. The two completely sequenced Co6304 variants were equally dominant in the original host. However, only one of them was detected after transmission to sorghum, indicating a strong genetic drift and differentiation between host plants. These results provide evidence of extensive variation and complicated population structures among SCSMV genomes, and the data will be useful for the viral genotyping and control strategy development.

A novel root-knot nematode secretory protein interacts with a Golgi-associated host plant protein

B. XUE (1), G. Huang (2), T. Baum (3), R. Hussey (2), E. Davis (1)
(1) North Carolina State University, Raleigh, NC, U.S.A.; (2) University of Georgia, Athens, GA, U.S.A.; (3) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S141

Secreted parasitism proteins of the southern root-knot nematode (RKN), Meloidogyne incognita, play crucial roles in modulating successful host root infection and the formation of elaborate feeding cells called giant-cells. The 4D03 parasitism gene is expressed exclusively within the single dorsal esophageal gland secretory cell of RKN parasitic life stages and encodes a novel 191 amino acid secretory protein. The 4D03 genomic clone has three introns of 50bp, 107bp, and 47bp within the predicted ORF and this gene also exists in the genome of other RKN species. No phenotypic changes have been observed when 4D03 was expressed in *Arabidopsis thaliana*. Plant host-derived RNA interference targeted to the 4D03 transcript in transgenic Arabidopsis thaliana plants significantly reduced root galling induced by M. incognita. A positive interaction of the 4D03 protein with a Golgi-associated protein of tomato was detected in yeast two-hybrid screens and confirmed in subsequent co-transformation experiments. The interacting Golgi-associated protein has a predicted domain of the RGP (Reversibly Glycosylated Polypeptide) superfamily that is putatively involved in polysaccharide biosynthesis. The observed protein-protein interaction suggests that secreted RKN 4D03 protein may play a functional role in feeding site establishment by modulating wall synthesis in giant-cells.

Inoculum sources and spore survival in field soil of the sour rot pathogen, *Geotrichum candidum*

M. A. YAGHMOUR (1), R. M. Bostock (1), J. E. Adaskaveg (2), T. J. Michailides (3)
(1) UC Davis, Davis, CA, U.S.A.; (2) UC Riverside, Riverside, CA, U.S.A.; (3) University of California, Parlier, CA, U.S.A.
Phytopathology 100:S141

Post-harvest decay of peaches and nectarines complicates storage and shipment of fruits and has serious economic consequences. *Geotrichum candidum* causes sour rot of fruit both in the field and after harvest. In some packing houses, inferior but otherwise sound fruit treated with fungicide are typically culled and returned to orchards where they are discarded between rows in stone fruit fields. Sour rot developed significantly more on fruits culled than on fruit collected from the same orchards without any treatment. Depending on the variety, cull fruit with sour rot ranged from 3 to 26%. Since cull fruits are a source of inoculum in the field and we have shown that it is a source of propiconazole-insensitive isolates, a study on spore survival at two different soil depths was undertaken to assess the possibility of burying cull fruits in orchards as a measure to manage the disease. Over a one-year period, spore populations of *G. candidum* at 20 cm depth were significantly less than at 10 cm until March. At both depths, the population remained low after March, declining to less than 2% of the initial populations until the last sampling point in August. The results suggest that survival of spores from decaying fruits in the orchard is minimal if those fruits are crushed and plowed under, thus providing a safe way to dispose of culled fruit.

Incidence of *Agrobacterium tumefaciens* on walnut seeds used for rootstock production: Implications for crown gall management strategies

L. E. YAKABE (1), S. R. Parker (1), D. A. Kluepfel (1)
(1) USDA ARS Crops Pathology/Genetics Research Unit, Davis, CA, U.S.A.
Phytopathology 100:S141

Crown gall of walnut, caused by *Agrobacterium tumefaciens*, is traditionally managed by pre-plant soil fumigation since soil-borne, *A. tumefaciens* populations in newly planted orchards and nurseries are thought to be the primary inoculum source. Despite these practices, crown gall persists. The distribution of disease development in walnut nursery plots suggests *A. tumefaciens* may be seed-borne. Extensive surveys of two production nursery seed sources revealed *A. tumefaciens* is not present in or on seeds collected directly from the mother trees. However, if seeds contact the orchard floor, as is common in traditional harvesting practices, *A. tumefaciens* could be detected on seeds. The bacterium is limited to the husk material surrounding the walnut, is not found inside the hull, and prevalence of husk infestation increased with time of floor exposure. Artificial inoculation studies corroborate field survey findings. In order to develop management strategies to control seed-borne populations of *A. tumefaciens*, heat treatments (50, 55, and 60°C for 30, 45, and 60 min) are being evaluated. Disease incidence and germination rates resulting from these heat treated seeds will be discussed. In conjunction with our previous research demonstrating increased colonization of fumigated soils by *A. tumefaciens*, planting clean walnut seeds may be key when managing crown gall in walnut production.

The ColS/ColR two-component system is involved in virulence of citrus canker pathogen *Xanthomonas axonopodis* pv. *citri* 306

Q. YAN (1), N. Wang (1)
(1) Lake Alfred, FL, U.S.A.
Phytopathology 100:S141

Bacterial citrus canker disease, which is caused by *Xanthomonas axonopodis* pv. *citri*, is one of the most devastating diseases on citrus. To investigate the virulence mechanism of this pathogen, a *X. axonopodis* pv. *citri* mutant library was constructed by randomly mutagenesis using EZ::Tn5Tm. Multiple mutants were identified by screening the mutants using plant assay on grapefruit. Among the mutants with reduced virulence, four of them, named 256A10, 421E7, 386C6 and 417E10, are mutants of two-component system ColS/ColR (colS::Tn5 in mutants 256A10 and 421E7; colR::Tn5 in mutants 386C6 and 417E10). The pathogenicity of the mutants could be complemented using wild-type *colS* or *colR*, respectively. Collectively, our data demonstrated that the two-component system ColS/ColR is involved in the virulence of *X. axonopodis* pv. *citri*. How ColS/ColR system is involved in virulence of *X. axonopodis* pv. *citri* 306 is under further investigation.

Population dynamics of *Dactylella oviparasitica* in a *Heterodera schachtii* suppressive soil

J. YANG (1), J. O. Becker (2), J. Borneman (1)
(1) Department of Plant Pathology and Microbiology, University of California, Riverside, Riverside, CA, U.S.A.; (2) Department of Nematology, University of California, Riverside, Riverside, CA, U.S.A.
Phytopathology 100:S141

The initial objective of this study was to identify and isolate different strains of the fungus, *Dactylella oviparasitica*, from a *Heterodera schachtii*-suppressive soil. Toward this end, we endeavored to enumerate *D. oviparasitica* populations in *H. schachtii* cysts extracted from 16 regions of the field containing the suppressive soil. Using a sequence-selective quantitative PCR assay, *D. oviparasitica* was detected in only one of the 16 locations. *D. oviparasitica* was also not detected in the soil or purslane weed seeds (*Portulaca oleracea*) collected from this field. These results led to the development of a new hypothesis, which was that *D. oviparasitica* parasitizes newly formed females and eggs within the cysts, and that its population densities decrease after this food source (females and eggs in the cysts) has been utilized. To test this hypothesis, we performed root box experiments to collect freshly developed *H. schachtii* females and cysts from roots of sugar beets grown in the suppressive soil. When these samples were examined using the sequence-selective qPCR assay, high levels (as high as 10^9 copies per cyst) of *D. oviparasitica* were detected in most samples, providing evidence to support our hypothesis. Further examination of these samples by analysis of the rRNA intergenic transcribed spacer region identified two *D. oviparasitica* phylotypes. Future experiments will examine the relative abilities of the two strains to reduce *H. schachtii* populations.

A screening strategy of bacterial biocontrol agents towards Ralstonia wilt of ginger

W. Yang (1), J. GUO (1)
(1) Nanjing, PRC PEOPLES REP OF CHINA
Phytopathology 100:S141

Bacterial wilt caused by Ralstonia solanacearum (Smith) has become a severe problem on ginger in China, there are no effective measures to control this disease up to now. In order to develop a new method to control it, four hundred and twenty strains were isolated from different habitats of ginger including soil, stem and leaves, eighty-five of them were selected for BOX- and ARDRA-PCR based on the results of in-vitro antagonistic activity against R. solanacearum. An assessment of 19 antagonists that from different BOX clusters was established based on the activities of enzymes including protease, chitinase, cellulose, glucanase, and production of siderophores. Also they were chosen for greenhouse experiment on ginger. Their biocontrol efficacies were 26–69%. Efficacies more than 50% were achieved by Bacillus subtilis 1JN2, Myroides odoratimimus 3YW8, Bacillus amyloliquefaciens 5YN8 and Stenotrophomonas maltophilia 2JW6. This is the first report to use Myroides sp. and Stenotrophomonas sp. as biocontrol agents against ginger wilt caused by R. solanacearum, and these strains can be good candidates for further exploration to develop a commercial biocontrol agent.

Biological control of take-all of wheat by fluorescent *Pseudomonas* spp. from China

M. YANG (1), J. Guo (2), L. S. Thomashow (3), D. M. Weller (3)
(1) Nanjing Agriculture University and Washington State University, Pullman, WA, U.S.A.; (2) Nanjing Agricultural University, Nanjing, PRC PEOPLES REP OF CHINA; (3) USDA-Agricultural Research Service, Pullman, WA, U.S.A.
Phytopathology 100:S141

Take-all disease of wheat caused by the soilborne fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*) is one of the most important root diseases of wheat worldwide. In order to find biocontrol agents, bacteria were isolated from wheat at different stages of development in Hebei and Jiangsu provinces in China. Samples from rhizosphere soil, roots, stems and leaves were plated onto King's B agar. All of 105 isolates that inhibited *Ggt* in vitro were identified as *Pseudomonas* spp. by ARDRA analysis. Twenty-seven isolates, which represented 24 BOX-PCR groups, were selected for further study. Of these, 14 suppressed take-all in biocontrol assays under greenhouse conditions. The antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (DAPG) are major determinants of biocontrol of soilborne plant pathogens by fluorescent pseudomonads. Using PCR and primers specific for sequences within the biosynthetic operons responsible for production of these antibiotics, 4 of the 14 strains were found to produce PCA but none produced DAPG. High-pressure liquid chromatography (HPLC) analysis of 2-day-old cultures of these four strains confirmed the production of PCA. DNA sequence analysis within the *phzF* gene indicated that three strains were similar to the well-described PCA producer *P. fluorescens* 2-79. This is the first report of a 2-79-like strain isolated from outside of Washington State.

TMV inclusion bodies: Their formation and relationship to virus accumulation

X. YANG (1), R. S. Nelson (1), S. Bhat (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 100:S142

Many viruses produce intercellular inclusions during infection. Their relationship with the accumulation and intra- and inter-cellular movement of these viruses and to disease is not fully understood. Previous studies with TMV have identified a positive correlation between the size of an inclusion, referred to as the virus replication complex (VRC), induced in infected cells and the intensity of disease symptoms displayed by the infected host, *N. benthamiana*. In addition, there was a similar positive correlation between the size of these VRCs and the inclusions produced by ectopic expression of the 126 kDa protein of the virus fused with GFP (Liu et al. Plant Physiology 2005 138:1853-1865). We have since determined that the size of the VRCs can be modified by silencing specific host factors. For example, silencing rubisco activase expression results in greater accumulation of virus (up to 7 fold) and smaller, but much more numerous VRCs. In addition, using chimeric constructs of 126 kDa protein and a homolog from TVCV, the 125 kDa protein, which poorly forms inclusions, we determined that particular domains within the 126 kDa protein control formation of inclusions. In addition, the formation of inclusions is a temporal process involving multiple domains. TVCV induces milder symptoms than TMV in *N. benthamiana* and the relationship of symptoms and inclusion body formation will be discussed based on these results.

Effect of limestone on development of Verticillium wilt of spinach

W. YANG (1), A. Iglesia-Garcia (1), L. du Toit (2), B. Bluhm (1), J. Correll (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Mount Vernon, WA, U.S.A.
Phytopathology 100:S142

Verticillium wilt, caused by *Verticillium dahliae*, is an important vascular wilt disease of many agricultural crops and, more recently, has become problematic for spinach seed production. Development of Verticillium wilt is influenced by soil conditions such as pH, moisture, and temperature. In an effort to optimize greenhouse pathogenicity and virulence tests, the effect of limestone (5 g/L CaCO3) on the development, incidence, and severity of Verticillium wilt symptoms on spinach was evaluated. In addition, the infection process was monitored using wild-type isolates and genetically marked strains in the form of nitrate non-utilizing (nit) mutants. Limestone and no limestone treatments were evaluated and compared to a non-limed control treatment. Treatments consisted of amending potting mix with different rates of limestone application, or drenching the potting medium with a limestone solution. Potting mix pH was recorded before and after each experiment. Plants were inoculated by adding *V. dahliae* spores to the root plug and monitoring disease development. Both wild-type isolates and nit mutants caused symptoms of Verticillium wilt, and both types of isolates were recovered from inoculated plants. Limestone treatments caused a significant increase in disease severity of Verticillium wilt of spinach.

Use of formulation and adjuvant approaches to identify potential limitations in field performance of an experimental fungicide relative to benchmarks

C. YAO (1), J. Owen (1)
(1) Dow AgroSciences, Indianapolis, IN, U.S.A.
Phytopathology 100:S142

Compound A has shown a promising activity profile against a spectrum of fungal pathogens. In preliminary greenhouse bio-assays using technical material and a high-volume spray format, it was 4-fold more potent as a protectant treatment, based on ED50 values, than azoxystrobin and epoxiconazole vs. wheat brown rust (*Puccinia recondita*, PUCCRT), while its curative potency was 3-fold weaker than azoxystrobin, and 10-fold weaker than epoxiconazole. Rust activity of Compound A formulated as a base SC was further compared to the commercial formulations of azoxystrobin (Amistar®) and epoxiconazole (Opus®) in low-volume spray applications. Compound A showed better protectant rust activity than both commercial products, while curative activity was better than Amistar® but weaker than Opus®. However, PUCCRT efficacy of Compound A was significantly weaker than Amistar® and Opus® in field trials. This discrepancy between greenhouse and field efficacies was partially attributed to the presence of a constant rate (0.1%) of an alcohol ethoxylate adjuvant in the spray solutions of Compound A. When it was compared to similar base SC of azoxystrobin and epoxiconazole and the same adjuvant added to 0.1% in all spray solutions, curative activity of Compound A vs. PUCCRT was at least 26-fold less than the two commercial fungicides, even though the protectant activities of all three fungicides were comparable. Data generated using the latter approach may improve predictions of field performance of experimental fungicides.

Soil amendments with *Brassica* cover crops for control of Phytophthora blight on squash

J. YIN (1), D. Koné (1), M. Purvis (1), K. L. Jackson (1), A. S. Csinos (1), P. Ji (1)
(1) Department of Plant Pathology, University of Georgia, Tifton, GA, U.S.A.
Phytopathology 100:S142

Phytophthora blight caused by *Phytophthora capsici* has become an increasing concern in vegetable production in Georgia and several other states in the U.S. Cover crops that produce general biocides, such as mustard, collards, canola, and other *Brassica* species, were studied under greenhouse and field conditions to determine host status and evaluate the efficacy to suppress *P. capsici*. In greenhouse studies, disease incidence on squash was significantly reduced by soil amendment with mustard leaves or roots. Soil amendments with leaves of canola or carrot also reduced disease incidence significantly. In field studies, cover crops were grown in winter and incorporated into soil in spring and squash seedlings were transplanted after soil amendment. Mustard and canola provided the greatest disease reduction while radish and collards reduced disease to a lesser extent. None of the cover crops showed symptoms when leaves or roots of the crops were inoculated with *P. capsici* and most of the cover crops did not appear to be a host of the pathogen. The results indicated that some *Brassica* cover crops had the potential to inhibit *P. capsici* and suppress disease development on squash plants when used as soil amendments.

Comparison of bacterial communities from inside and outside of Rhizoctonia bare patches in wheat

C. Yin (1), S. H. Hulbert (1), K. L. Schroeder (2), O. Mavrodi (1), D. Mavrodi (1), A. Dhingra (3), T. C. PAULITZ (2)
(1) Dept. of Plant Pathology, Washington State University, Pullman, WA, U.S.A.; (2) USDA-ARS, Root Disease and Biological Control Research Unit, Pulllman, WA, U.S.A.; (3) Dept. of Horiculture and Landscape Architecture, Washington State University, Pullman, WA, U.S.A.
Phytopathology 100:S142

Rhizoctonia solani AG-8 causes distinct patches of stunted wheat in the field. Bacterial communities from bulk soil and rhizospheres of wheat were analyzed with pyrosequencing. Replicated samples were taken from inside and outside of patches; and from patches that had recovered the previous 1–2 years. Pyrosequencing was performed on amplified products from primers designed to the V3 hyper-variable region of bacterial 16S rDNA. Between 2070 and 5746 sequences of >150 bp were obtained from each sample, and these were assembled into 345 OTUs, with 151 identified to the genus level. Community diversity was higher and abundance was lower in the bulk soil compared to the rhizosphere soil. In the bulk soil, abundance was highest inside the patches. *Chitinophaga* and *Acidobacteria* GP 3 were the most abundant genera, followed by Enterobacteriaceae, *Pseudomonas*, *Pedobacter*, *Variovorax*, *Sphingomonas*, *Solirubrobacter*, *Nitriliruptor* and TM7. *Flavobacterium* and a single OTU in the family Enterobacteriaceae and the order Sphingobacteriales were more frequent in the rhizospheres of plants inside the patch compared to outside or recovered patches, and this was confirmed by real-time quantitative PCR. *Dyella* and *Acidobacteria* GP 7 were more frequent in recovered patches. These results show that the rhizosphere community on wheat plants in diseased patches is distinct from healthy plants, and this technique may be useful for finding organisms associated with disease suppression.

Molecular characterization of boscalid resistance in field isolates of *Botrytis cinerea* from apple in Washington State

Y. YIN (1), Y. Kim (1), C. Xiao (1)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.
Phytopathology 100:S143

To monitor boscalid resistance in the field, *Botrytis cinerea* isolates obtained from 5 commercial apple orchards were screened for resistance to boscalid using a conidial germination assay at the discriminatory concentration of 5 µg/ml. Of the 220 isolates tested, 42 were resistant to boscalid. There was cross resistance between boscalid and either carboxin or penthiopyrad. Analysis of partial sequences of the iron-sulphur subunit of succinate dehydrogenase gene (*BcSdhB*) from 13 boscalid-resistant and 9 -sensitive isolates showed that point mutations in the *BcSdhB* gene leading to amino acid substitutions at the codon position 272 from histidine to either arginine (H272R) or tyrosine (H272Y) were associated with boscalid resistance. Allele-specific PCR analysis of the 66 boscalid-resistant isolates (including 24 additional isolates obtained from decayed apples) showed that 46 had the point mutation H272R and 19 exhibited the point mutation H272Y, but one resistant isolate gave no amplification product. Analysis of *BcSdhB* sequence of this isolate revealed a different point mutation at the codon position 225, resulting in a substitution of proline (P) by phenylalanine (F) (P225F). A multiplex allele-specific PCR assay was developed to detect point mutations at the position 272 in *BcSdhB* conferring resistance to boscalid in a single PCR amplification. There was no correlation between types of point-mutation in *the BcSdhB* gene and levels of boscalid resistance in the resistant isolates.

Characterization of pyraclostrobin resistance and detection of the Bcbi-143/144 intron in the cytochrome *b* gene in *Botrytis cinerea* isolates from apple

Y. YIN (1), Y. Kim (1), C. Xiao (1)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.
Phytopathology 100:S143

To monitor resistance to the QoI fungicide pyraclostrobin (PYR) in the orchard, *Botrytis cinerea* isolates obtained from 5 orchards where PYR had been used for 4 consecutive years were screened for resistance to PYR in a mycelial growth assay on potato dextrose agar amended with PYR at the discriminatory concentration of 5 µg/ml and salicylhydroxamic acid at 100 µg/ml, which was used to inhibit the alternative oxidase respiration. Of the 220 isolates tested, 43 (19.5%) were resistant to PYR. All resistant isolates were highly resistant to PYR with resistance factors >1000. Cross-resistance was observed between PYR and two other QoIs azoxystrobin and trifloxystrobin. Analysis of partial sequence of the cytochrome *b* gene (*cytb*) and molecular diagnosis based on an allele-specific PCR showed that all PYR-resistant (PYR-R) isolates had the same mutation leading to the substitution of glycine by alanine at codon position 143 in *cytb* (G143A). Structural analysis of *cytb* indicated that all PYR-R isolates did not have the Bcbi-143/144 intron. Of the 123 PYR-sensitive (PYR-S) isolates collected from 4 major apple producing regions in Washington State, only 17 (13.8%) had the Bcbi-143/144 intron in *cytb*, indicating a high inherent-risk for development of QoI resistance among the PYR-S isolates as the presence of the Bcbi-143/144 intron in *cytb* is believed to prevent the occurrence of G143A mutation-mediated QoI resistance.

The source of polypeptone in culture medium affects lipopeptide production by *Bacillus subtilis*

K. YOKOTA (1), E. Miwa (1), K. Higuchi (1)
(1) Tokyo University of Agriculture, Tokyo, JAPAN
Phytopathology 100:S143

Iturin and surfactin are antimicrobial lipopeptides thought to play a key role in antimicrobial activity of biological control strains of *Bacillus* spp., including *B. subtilis*. We report here that the source of polypeptone used in submerged culture medium influences production of iturin and surfactin. These lipopeptides were produced if the polypeptone source was either crude soybean cake or potato, but not if the source was purified soybean protein, milk casein, or fish meat. The data suggest that the lipopeptide-inducing factor in polypeptone from crude soybean is lost after protein purification, and that it is not present in polypeptone from certain animal sources.

Effect of solar radiation on disease severity of soybean rust

H. M. YOUNG (1), D. F. Narvaez (2), J. J. Marois (3), D. L. Wright (3)
(1) University of Florida, Gainesville, FL, U.S.A.; (2) Monsanto, St. Louis, MO, U.S.A.; (3) University of Florida, Quincy, FL, U.S.A.
Phytopathology 100:S143

Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, is the most damaging fungal disease of soybean. While it is known that solar radiation can reduce SBR spore survival, increase canopy temperature and reduce humidity, limited information is available on how solar radiation affects SBR progress within the soybean canopy. Such information can aid in accurate SBR prediction models. To manipulate light penetration into soybean canopies shade structures (using 30, 40, and 60% shade cloth) were constructed over soybeans and weekly evaluations of severity of lower, middle, and upper canopies were recorded, as well as, daily environmental conditions. Detached leaf assays assessed cuticular wax amount and the susceptibility of leaves in the lower, middle, and upper canopies. The experiment was conducted over 2 years, with 2 plantings each year, and 3 replications each planting. Temperature and relative humidity were affected by shade cloth, regardless of thickness. A trend of decreasing cuticular wax from upper to lower canopy level was observed, for all treatments. Greater disease severity occurred in 40 and 60% shaded field plots and in detached leaf assays of middle and upper canopy leaves from 40 and 60% shaded plots. These results provide an understanding of the effect solar radiation has on the progression of SBR within soybean canopy.

Selection of single chain variable fragments (scFv) against *Xylella fastidiosa* subsp. *pauca* by phage display

Q. YUAN (1), R. Jordan (1), R. H. Brlansky (2), O. Minenkova (3), J. Hartung (1)
(1) USDA ARS MPPL, Beltsville, MD, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.; (3) Sigma-tau Pharmaceuticals, Rome, ITALY
Phytopathology 100:S143

Xylella fastidiosa is a gram-negative member of the gamma proteobacteria. *Xylella fastidiosa* subsp. *pauca* causes citrus variegated chlorosis in Brazil and enjoys 'select agent' status in the United States. Antibody based detection assays are commercially available for *Xylella fastidiosa*, and are effective at the species, but not at the subspecies level. We have made a library of scFv antibody fragments directed against *Xylella fastidiosa* subsp. *pauca* strain 9a5c (citrus) by using phage display technology. BALB/c mice were immunized with 9a5c bacteria at a concentration of 10^8 cfu/100 ul buffer. mRNA from the spleens of the immunized mice was purified and converted into cDNA. Antibody gene repertoires 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 then the genes of the scFv library were ligated into the phage vector pKM19. The library contained 1.2×10^7 independent clones with full-length scFv inserts. In each of 3 cycles of affinity-selection with 9a5c, about 1.0×10^{12} phage were used for panning with 4.1×10^6, 7.1×10^6, 2.1×10^7 phage recovered after the first, second and third cycles respectively. 66% of clones from the final library bound *Xylella fastidiosa* 9a5c. Some of these phage expressing scFv antibodies recognized strain 9a5c but did not recognize *Xylella fastidiosa* strains that cause Pierce's disease of grapevine.

Development of conventional monoclonal antibody and recombinant antibody (scFv) against *Candidatus* Liberibacter asiaticus

Q. YUAN (1), R. Jordan (1), R. C. Bohannon (2), R. H. Brlansky (3), O. Minenkova (4), J. S. Hartung (1)
(1) USDA ARS MPPL, Beltsville, MD, U.S.A.; (2) Agdia, Elkhardt, IN, U.S.A.; (3) University of Florida, Lake Alfred, FL, U.S.A.; (4) Sigma-tau Pharmaceuticals, Rome, ITALY
Phytopathology 100:S143

A non-culturable member of the alpha-proteobacteria, 'Ca. Liberibacter asiaticus', is consistently associated with huanglongbing (HLB) disease. This bacterium is transmitted by citrus psyllids and grows systemically in infected citrus phloem tissues. Control of the disease requires effective, convenient and inexpensive methods for detection of the pathogen in infected plants and insects. Antibodies are the most widely used tool to detect pathogens, and they are also uniquely useful as experimental reagents. Therefore antibodies against the HLB pathogens would greatly aide detection of the pathogen and eventual control of this disease. Extracts of psyllids fed on HLB infected citrus in Florida were assayed individually for 'Ca. Liberibacter asiaticus' by q-PCR. Extracts with more than 108 'Ca. Liberibacter asiaticus'/100 ul were used to immunize BALB/C mice. Monoclonal antibodies were made by spleen/myeloma fusion and screened against both plant extracts containing 'Ca. Liberibacter asiaticus' and against outer membrane protein (OMP) purified from Escherichia coli cells expressing the cloned gene. A recombinant library of scFv antibodies also was made using the phage vector pKM19 to clone cDNAs prepared from the mRNA isolated from the mouse spleens. This scFv library in recombinant phage is currently being screened against extracts of plants infected with high concentrations of 'Ca. Liberibacter asiaticus' as well as against purified outer membrane protein from 'Ca. Liberibacter asiaticus'.

Foliar symptoms expression and early infection of soybean sudden death syndrome

M. L. ZACCARON (1), X. Yang (1), S. S. Navi (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S144

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is an important root disease that can express foliar symptoms leading to defoliation and yield reduction. Our earlier report showed that root cap is one of the penetration sites of infection for *F. virguliforme*, which will be followed by foliar symptoms in soybean. There is not much information about infection process of SDS and how environmental conditions influence the process and subsequent foliar symptoms development. The objective of this study was to investigate infection sites on soybean roots leading to foliar symptoms expression. Seeds were germinated using paper towel method at room temperatures; radicles of 72-h were inoculated with 20 µl of conidial suspension *F. virguliforme* using inoculation loop and micropipette methods under sterile conditions. Three sites of infection on different locations were examined. After inoculation germinating seeds were transplanted to cones filled with sterile potting mixture. Rhizosphere temperature was controlled with water bath at (20°C). Plants were evaluated three weeks after transplanting. Our preliminary results indicate that plants inoculated at root tip had higher incidence than plants inoculated at other sites. The incidence was even higher when the inoculation loop was used, suggesting mechanical wounding early at soybean planting may be critical to foliar symptom expression.

Transposon mutagenesis of *Pantoea ananatis*: Isolation and characterization of a Tn*5*-induced mutant with reduced virulence to onion

A. M. Zaid (1), J. M. Bonasera (1), S. V. BEER (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S144

Center rot of onion, caused by *Pantoea ananatis*, is a disease of increasing concern to onion growers in New York State. We recently isolated both pathogenic and non-pathogenic strains of *P. ananatis* from New York onions. The strains are indistinguishable from each other based on numerous biochemical assays. OC5a, a highly virulent New York strain was mutated using transposon Tn*5*. Two-thousand mutants were screened for virulence in small onion bulbs (sets). Southern blot analysis of 27 mutants revealed that Tn*5* has a strong insertion-site preference in the *P. ananatis* genome. Thus, not surprisingly, only a single less virulent mutant was found; it contained two additional insertions of Tn5. These insertion sites will be characterized as to the genes they may have inactivated and the potential role of the disrupted genes in virulence to onion. We seek to identify genes of *P. ananatis* that are involved in virulence to advance our understanding of the genetic basis for pathogenesis and to provide molecular markers for pathogenic strains that would likely be useful for future diagnostic and epidemiological studies.

Effect of temperature on latent period of Stagonospora nodorum blotch in winter wheat under field conditions

A. D. ZEARFOSS (1), C. Cowger (2), P. S. Ojiambo (1)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S144

Stagonospora nodorum blotch (SNB) is caused by *Stagonospora nodorum* (teleomorph *Phaeosphaeria nodorum*) and yield losses from severe disease epidemics can be as high as 50%. To establish a model for SNB development based on the effects of temperature on pathogen latent period relative to the host, batches of two winter wheat cultivars (AGS 2000 and USG 3209) were inoculated with pycnidiospores of *S. nodorum* at weekly intervals from February 2009 to June 2009. After an incubation period of 72 h, plants were exposed to field conditions where temperatures ranged from –6.6°C to 35.8°C with a mean batch temperature of 9.7°C to 23.7°C. Latent period expressed as the time from inoculation until the first visible symptoms, ranged from 8 to 34 days. A shifted cumulative gamma distribution model with a base temperature of 0.5°C best described the relationship between number of lesions with pycnidia and accumulated thermal time. When defined as time to 50% of the maximal lesions with pycnidia, latent period was estimated as 297 and 313 degree-days above the base temperature of 0.5°C for USG 3209 and AGS 2000, respectively. The relationship between the inverse of latent period, defined as time to 50% maximal lesions with pycnidia, and the mean batch temperature was best described using a linear model ($r^2 = 0.93$, $P < 0.001$). This study provides data that link wheat growth with SNB progress and will facilitate the construction of disease development models for use in timing of fungicide application.

Survey of *Stagonospora nodorum* toxins and wheat sensitivity genes in the southeastern U.S.

A. D. ZEARFOSS (1), T. L. Friesen (2), P. S. Ojiambo (1), C. Cowger (3)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) USDA-ARS, Department of Plant Pathology, North Dakota State University, Fargo, ND, U.S.A.; (3) USDA-ARS, Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S144

Stagonospora nodorum blotch (SNB), caused by the necrotrophic fungal pathogen *Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*), is among the most ubiquitous diseases of winter wheat in the U.S. The disease is favored by warm, moist weather conditions prevalent in the southeastern U.S., and can cause substantial reductions in yield and test weight. Host resistance is the most effective and economically viable option for SNB control. Recent discovery of several host-selective toxins (HSTs) produced by *S. nodorum*, and their corresponding host sensitivity genes, has offered new resources for SNB resistance breeding. Knowledge of HSTs and toxin-sensitivity genes in regionally adapted wheat breeding materials can aid small grains breeding programs in the southeast. Fifty-four isolates of *S. nodorum* collected from wheat debris from nine states in the southeastern U.S. were used to obtain culture filtrates for host infiltration. Twenty-four advanced soft red winter wheat (SRWW) lines with varying levels of resistance to SNB were chosen from nine southeastern states. Three toxin controls, three fungal isolate controls and six differential wheat lines were also used. Each cultivar × isolate reaction was assessed 3, 5 and 7 days after inoculation and scored as positive, intermediate or negative. We will report HSTs found in *S. nodorum* isolates and postulate sensitivity genes in elite SRWW lines and released varieties commonly grown in the southeastern U.S.

The Irony of Silicon: Accumulation in a non-accumulator induced by TRSV

W. L. ZELLNER (1), J. Frantz (2), S. M. Leisner (1)
(1) University of Toledo, Toledo, OH, U.S.A.; (2) U.S. Department of Agriculture-Agricultural Research Service, Toledo, OH, U.S.A.
Phytopathology 100:S144

While silicon protects a variety of plants against fungal and bacterial pathogens, silicon-aided resistance to viral pathogens is as unclear as the amount of silicon required to aid in plant defenses. Therefore, the effects of and accumulation of silicon on *Tobacco ringspot virus* (TRSV) were investigated in the "low-accumulator", *N. tabacum*. Plants were grown hydroponically and prior to inoculation the levels of soluble silicon were increased. TRSV symptoms on *N. tabacum* treated with increased silicon typically took two days longer to appear and covered less leaf area then plants grown under control conditions. Furthermore, preliminary studies using TMV show no change in the onset or distribution of symptoms in *N. tabacum*, suggesting that the beneficial effects of silicon are pathogen-specific. ICP analysis of leaf and root tissue indicated a significant accumulation of silicon in leaves of TRSV-infected plants grown under increased silicon compared to leaves of mock-inoculated plants. However, root silicon levels were elevated for all plants supplemented with silicon, independent of TRSV infection. This suggests that *N. tabacum* accumulates silicon in root tissue which is then released during TRSV infection, permitting acquisition by leaves. Salicylic acid (SA) treatment of *N. tabacum* also resulted in an increase in silicon accumulation in upper leaves compared to control plants, suggesting that SA may play a role in the signaling pathway.

Regulation of *Dickeya dadantii* type III secretion system by polynucleotide phosphorylase

Q. ZENG (1), A. Ibekwe (2), C. Yang (1)
(1) University of Wisconsin-Milwaukee, Milwaukee, WI, U.S.A.; (2) USDA-ARS, US Salinity Laboratory, Riverside, CA, U.S.A.
Phytopathology 100:S144

The type III secretion system (T3SS) is an essential virulence factor of the phytopathogenic bacterium *Dickeya dadantii*. In *D. dadantii*, the transcription of T3SS structural and effector genes is positively regulated by the master regulator, HrpL. Expression of *hrpL* is regulated at the transcriptional level by RpoN and post-transcriptionally by RsmB, a regulatory small RNA, which enhances *hrpL* mRNA stability. Polynucleotide phosphorylase (PNPase) is one of the major exoribonucleases in bacteria and plays important roles in general mRNA degradation, tRNA processing, and sRNA turnover. In this study, we showed that PNPase down-regulates the transcription of T3SS genes. This negative regulation of T3SS by PNPase occurs through the repression of *hrpL* expression. By decreasing *rpoN* mRNA stability, PNPase down-regulates the transcription of *hrpL*, which leads to a reduction in T3SS gene expression. Moreover, we have found that PNPase down-regulates T3SS by reducing *hrpL* mRNA stability. Our results suggest that PNPase decreases the amount of functional RsmB transcripts, which could result in the reduced *hrpL* mRNA stability.

Genotypic and phenotypic diversity of *Pyricularia oryzae* in the contemporary rice blast pathogen population in Arkansas

L. ZHAI (1), C. Feng (1), R. Cartwright (1), J. Correll (1)
(1) University of Arkansas, Department of Plant Pathology, Fayetteville, AR, U.S.A.
Phytopathology 100:S145

Rice blast, caused by *Pyricularia oryzae*, is one of the most economically important diseases of rice worldwide. It is also one of the most important rice diseases in Arkansas. The objective of this study was to examine the genotypic and phenotypic diversity of *P. oryzae* in the contemporary population in Arkansas. The weather during the 2009 rice season was particularly conducive for disease development. Over 500 isolates were recovered from symptomatic rice cultivars in Arkansas during the 2009 rice season. Theses isolates were evaluated for vegetative compatibility, MGR586 (Magnaporthe grisea repeat sequence) DNA fingerprint diversity, and virulence on a set of 30 commercial cultivars or advanced breeding lines. Although four VCG groups (US-01 to US-04) have been identified among contemporary and archived isolates of *P. oryzae* in Arkansas, only three VCG groups (US-01, US-02 and US-04) were identified in our 2009 collection. VCG US-01 remains predominant and over 50% of these isolates recovered from 2009 belonged to VCG US-01. Pathogenicity test of a subset isolates on the commercial cultivars and advanced breeding lines indicated that there was considerable virulence diversity present both between and among isolates in a VCG or MGR fingerprint group. The genetic population analysis will provide us a better understanding of host coevolultion in plant pathosystems and provide us direction for effectively managing rice blast.

Bactericidal activities of antimicrobial molecules against huanglongbing-associated 'Candidatus Liberibacter asiaticus' in the diseased periwinkle

M. Zhang (1), Y. Duan (2), C. A. POWELL (3)
(1) University of Florida, Ft. Pierce, FL, U.S.A.; (2) United States Department of Agriculture-ARS-USHRL, Fort Pierce, FL, U.S.A.; (3) IRREC-IFAS University of Florida, Fort Pierce, FL, U.S.A.
Phytopathology 100:S145

Candidatus Liberibacter is a phloem-limited, gram-negative, fastidious bacterium that is associated with the citrus Huanglongbing (HLB) disease. Periwinkle was used as a model plant to test molecules for bactericidal activity against Liberibacter. Liberibacter-infected, 4-cm periwinkle cuttings were soaked in solutions containing one of several molecules including a biocide (DBNPA), two peptides (D2A21 and D4E1), a fungicide (Zineb) and SAR substances (SA, antiguard and ortho-phenylphenol). Cuttings prior to treatment and their regenerated plants at 2 and 3 months post treatment were analyzed for Liberibacter by quantitative real-time (q)PCR with primer set HLBas/HLBr/HLBp. The peptides consistently reduced the bacteria titers with an average Ct values for both peptides of 34.87 ± 4.45 and 37.00 ± 1.77 by qPCR in the regenerated plants at 2 and 3 months post treatment respectively, compared to the water-control (22.59 ± 4.96 and 24.89 ± 1.39). Biocide (DBNPA) could also suppress the Las bacteria. The fungicide zineb and three SARs (SA, antiguard and ortho-phenylphenol) were not effective in controlling Liberibacter bacteria. Whether treated with zineb or not, the Liberibacter bacteria can keep reproducing. The Ct value was lower in the zineb-treated, regenerated plants than those treated with peptides. Because peptides were very effective but expensive in eliminating the bacterium in the Liberibacter-infected citrus in the field, they can be expressed in the transgenic citrus to control citrus HLB disease.

Baseline sensitivity of *Cercospora sojina* to azoxystrobin, trifloxystrobin, and pyraclostrobin

G. ZHANG (1), D. V. Phillips (2), C. A. Bradley (1)
(1) University of Illinois, Urbana, IL, U.S.A.; (2) University of Georgia, Griffin, GA, U.S.A.
Phytopathology 100:S145

Frogeye leaf spot (FLS) is a disease of soybean caused by Cercospora sojina. Quinone outside inhibitor (QoI) fungicides such as azoxystrobin, trifloxystrobin, and pyraclostrobin are applied to manage FLS. A total of 58 "baseline" isolates collected from 9 states prior to QoI fungicide registration in the U.S. was tested with an in-vitro spore germination assay to determine the effective fungicide concentration at which 50% of conidial germination was inhibited (EC50). The effect of salicylhydroxamic acid (SHAM) on conidial germination also was tested to determine if C. sojina can use the alternative respiration pathway to bypass the effect of the fungicides. Significantly greater (P 0.05) EC50 values were observed when media was amended with azoxystrobin alone compared with media amended with both azoxystrobin and SHAM. This indicates that C. sojina may use alternative respiration to overcome the effect of QoI fungicides in-vitro, and that the inclusion of SHAM is necessary when testing the sensitivity of C. sojina isolates to QoI fungicides. Baseline isolates had EC50 values for pyraclostrobin, trifloxystrobin, and pyraclostrobin that ranged from 0.0029 to 0.0323, 0.00018 to 0.00311, and 0.00014 to 0.00076 ug/ml, with means of 0.0127, 0.0012 and 0.00027 ug/ml, respectively. The establishment of these baseline QoI sensitivity values is an important first step in developing a fungicide resistance monitoring program for C. sojina.

Evaluation of biologically-based products for managing bacterial spot disease of tomato

S. ZHANG (1), T. L. White (1), Y. Fu (1)
(1) TREC - University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S145

Bacterial spot, caused by *Xanthomonas* spp., is a devastating disease of tomato in Florida and worldwide. Despite the tremendous efforts towards managing this disease, it still remains a major challenge in the Southeastern states. Greenhouse experiments were conducted to evaluate efficacy of six biologically-based products alone or in combination with Actigard®, an inducer of systemic acquired resistance (SAR) registered for tomato. Beginning 1 week after transplanting into 4-inch plastic pots, plants were sprayed four times either with suspensions of biological products alone or in combination with Actigard® at weekly intervals. Kocide 3000 plus Manzate (K+M) was used as a standard chemical control, and water sprays were served as the nontreated control. Tomato plants were spray-inoculated with suspensions of *X. perforans* (2×10^7 CFU/ml) 3 days after the last treatment and placed on a greenhouse bench for 10 days when the disease was rated on a 0–5 scale. The products HMO 736 and Companion each alone or in combination significantly ($P < 0.05$) reduced the disease severity compared to the nontreated control, and was as effective as K+ M. Other products in combination with Actigard® resulted in significantly lower disease than each of these biologicals alone. Combined treatments numerically reduced the disease compared to Actigard® alone. Results indicate that these biologically-based products could potentially be incorporated into integrated management programs for control of bacterial spot on tomato.

Management of powdery mildew on squash with biologically-based products

S. ZHANG (1), T. L. White (1)
(1) TREC - University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S145

Powdery mildew, caused by *Sphaerotheca xanthii*, is a serious disease of cucurbits worldwide. Many fungicides have been registered for this disease control. However, management of fungicide resistance in the pathogen is a challenge. Efficacy of six biologically-based products, alone or in alternation with a conventional fungicide, against powdery mildew was evaluated on squash under greenhouse and field conditions. In greenhouse experiments, products Regalia and BU EXP 1216 S each significantly ($P < 0.05$) reduced the disease severity compared to the nontreated control, and they were as effective as Procure, the standard fungicide treatment. The products HMO 736 and BU EXP 1216 S, each in alternation with Procure, consistently resulted in significantly lower disease than the nontreated control. Similarly, Regalia, Actinovate, Companion, and BU EXP 1216 C each when alternated with Procure provided significant protection in two out of three experiments. More interestingly, Regalia and HMO 736 had a synergistic effect when alternated with Procure in one of the experiments. In the field trial, the biological products applied individually or in alternation with Procure significantly reduced powdery mildew severity at the early stage of disease development and significantly improved the control efficacy of the biologicals and Procure at the late stage. These data suggest that these biologically-based products alternated with Procure could be used for managing powdery mildew of squash in Florida.

Organization and structure of two stable Ca. Liberibacter asiaticus prophage lysogens that become lytic in plant infections

S. ZHANG (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S145

Citrus Huanglongbing (HLB), caused by Candidatus Liberibacter asiaticus (Las), is a psyllid transmitted, lethal disease of citrus now found widespread in Florida citrus growing regions. The recently published Las strain psy62 genome, obtained from a single psyllid (GenBank NC_012985.2), revealed prophage DNA integrated in the genome. Phage have not been previously associated with HLB. A fosmid DNA library from curated Las strain UF506, isolated from an infected Florida citrus tree, seemed surprisingly biased towards phage DNA inserts. Two highly related circular phage genomes (SC1 and SC2) were assembled and annotated from the UF506 library, including 5 additional genes not previously identified in psy62. Extensive Southern blot and PCR analyses were used to: 1) confirm the presence of lytic cycle SC1 and SC2 phage in Las infected citrus and periwinkle but not in Las infected psyllids; 2) confirm the SC1 and SC2 circular phage genomic assemblies; 3) map the cos sites of both phage, and 4) determine the genomic DNA

integration sites and gene order of both SC1 and SC2 prophage. Semi-quantitative RT-PCR revealed that the copy number of (lytic cycle) SC-1 in infected citrus and periwinkle averaged 10X and 20X higher, respectively, than in (lysogenic cycle) infected psyllids. The SC-2 phage DNA appeared at a level 2-3X higher in planta than in psyllids. Phage particles associated with Las were found in the pholem of infected periwinkles by transmission EM.

Antagonistic role of ethylene and abscisic acid in mediating rice sheath blight resistance

J. Zhang (1), D. Park (2), Y. YANG (1)
(1) Department of Plant Pathology and Huck Institute of Life Sciences, Pennsylvania State University, University Park, PA, U.S.A.; (2) National Yeongnam Agricultural Research Institute, Rural Development Administration, Milyang, KOREA
Phytopathology 100:S146

Sheath blight, caused by *Rhizoctonia solani*, is one of the most important rice diseases in the U.S. and around the world. However, little is known about the molecular mechanism and defense signaling pathways that mediate basal and partial resistance against this necrotrophic pathogen. In this study, we attempt to determine the role of ethylene (ET) and abscisic acid (ABA) in sheath blight resistance using transgenic rice lines defective in hormone pathways as well as exogenous treatments of hormones or their chemical inhibitors. Treatments of Nipponbare cultivar with 0.2 mM ethephon, which releases ET, significantly reduced lesion length and disease severity. By contrast, treatments with 0.1 mM ABA greatly increased lesion length on both Nipponbare and resistant cultivar Jasmine 85. Application of fluridone (an ABA biosynthesis inhibitor) on Nipponbare rice markedly reduced lesion length, whereas treatments with aminooxyacetic acid and 1-MCP (ET biosynthesis and signaling inhibitors, respectively) increased lesion length on Nipponbare and Jasmine 85. Furthermore, transgenic rice lines defective in ET signaling exhibited increased susceptibility to *Rhizoctonia solani* infection, whereas transgenic lines with an increased ET level showed enhanced sheath blight resistance. Together, these results suggest that basal and partial resistance to rice sheath blight is positively regulated by ET biosynthesis and signaling, but negatively modulated by ABA pathway.

Biological control of banana wilt

Y. Zheng (1), J. GUO (1)
(1) Nanjing, PRC PEOPLES REP OF CHINA
Phytopathology 100:S146

Banana wilt is one of the most destructive diseases on banana production worldwide, caused by Fusarium oxysporum f. sp. cubense (Foc). No effective control measure for Fusarium wilt has been found other than the use of resistant cultivars. In our study, 539 bacterial strains were isolated from soil, roots, pseudostem, corn and leave of banana plants. According to their in vitro antagonistic activities against Foc race 1, race 4 and their enzyme activities including protease, chitinase, and cellulase, 177 strains with obvious activities were selected for diversity study using amplified ribosomal DNA restriction analysis (ARDRA) and BOX-PCR. Through analysis of the fingerprints, 22 strains from different groups were selected for greenhouse experiments on banana. These 22 antagonists belonged to genus of Bacillus, Serratia, Pantoea, Enterobacter, Burkholderia, Stenotrophomonas and Lysinibacillus based on the 16S rRNA gene sequences, were confirmed in greenhouse study with the biocontrol efficacy of 38.64–84.09%, and increased biomass by 15.92–64.73%. Finally, 5 potential biological control agents appearanced in field experiment in Guangdong Province, and achieved biocontrol efficacy of 35.96–69.65%.

Complete genome sequence of *Capsicum chlorosis virus* from *Phalaenopsis* orchid and prediction of the unexplored genetic information of tospoviruses

Y. Zheng (1), C. Chen (1), F. JAN (1)
(1) National Chung Hsing University, Taichung, TAIWAN
Phytopathology 100:S146

Phalaenopsis orchids are popular ornamentals all over the world. A tospovirus, *Capsicum chlorosis virus* (CaCV-Ph) had been identified to cause the chlorotic ringspots on leaves of *Phalaenopsis* orchids in Taiwan. The tripartite genome of CaCV-Ph was found to contain 3608, 4848 and 8916 nts of S, M and L RNAs, respectively. The phylogenetic relationship of the nucleocapsid (N) protein indicated that CaCV-Ph was a member of *Watermelon silver mottle virus* (WSMoV) serogroup in the genus *Tospovirus*. Based on the relations among the nonstructural protein (NSs), glycoprotein (GnGc), thrips genera, host and geographical distribution, they could be classified into two major types: WSMoV-*Thrips*-Asian and *Tomato spotted wilt virus* (TSWV)-*Frankliniella*-EuroAmerican. The proline (P_{459}) of all tospoviral Gn proteins was indispensable and the RGD motif maintained by partial tospoviruses may not involve in the viral transmission. A RdRp catalytic domain found in the conserved region of L protein may recognize the typically conserved sequences on the 5′ and 3′ terminal regions (5′ AGAGCAAU 3′). In this study, the genetic information of CaCV-Ph was investigated by bioinformatic analyses for further investigation of CaCV-Ph properties.

Occurrence of a rice dwarf disease in China caused by Southern rice black-streaked dwarf virus, a new species in genus *Fijivirus*

G. ZHOU (1), Q. Wang (1), J. Wen (1), D. Xu (1)
(1) Laboratory of Plant Virology, South China Agricultural University, Guangzhou, PRC PEOPLES REP OF CHINA
Phytopathology 100:S146

Southern rice black-streaked dwarf virus (SRBSDV) is a newly proposed species in the genus *Fijivirus*, family *Reoviridae*. It was discovered on rice for the first time in 2001, in Yangxi county, Guangdong Province, China, and recorded previously as a new strain of *Rice black-streaked dwarf virus* (RBSDV) or RBSDV-2. During the past several years, it spread rapidly throughout southern China and northern Vietnam, and became one of the most important rice pathogens in those regions. In the 2009 crop season, 300 000 ha and 15 000 ha of rice was affectted in China and Vietnam, respectively, and more than 6 500 ha of crop failure was estimated. This virus is naturally transmitted by white-backed plnathopper (*Sogatella furcifera*), and infects rice, maize, barnyard grass (*Echinochloa crusgalli*), chinese sorghum (*Coix lacryma-jobi*), flaccid grass (*Pennisetum flaccidum*) and *Juncellus serotinus*. In artificial tests, rice plants at any growth stage could be infected and, the earlier they got infected, the more severe symptoms they developed. Symptoms include severe stunting, dark green leaf, small galls on stem, and rootlets and tillers on the upper nodes. A field survey at rice earing stage in October 2009 in Guangzhou, China, revealed that approximate 15% of plants were pronounced dwarf with all above symptoms and 11% showed slight dwaf with small galls on the stem and, moreover, 20% (10/50) symptomless plants were SRBSDV positive in RT-PCR detection.

Mechanism of suppression of a no-till hairy vetch cover crop on the spread of anthracnose on watermelon

X. ZHOU (1), K. L. Everts (2), C. Zhou (3)
(1) AgriLife Research, Texas A&M University System, Beaumont, TX, U.S.A.; (2) University of Maryland and University of Delaware, Salisbury, MD, U.S.A.; (3) University of Maryland, Salisbury, MD, U.S.A.
Phytopathology 100:S146

Anthracnose caused by *Colletotrichum orbiculare* is one of the most common and destructive diseases of watermelon worldwide. Previous experiments indicated that production of watermelon on a no-till hairy vetch (*Vicia villosa*) cover crop was effective in reducing anthracnose. The objective of this study was to examine the spread of anthracnose on watermelons when grown on vetch and other ground coverings to better understand the mechanism of action of disease suppression. Spread of watermelon anthracnose was evaluated in field plots over three years by assessing disease severity at a range of distances from an introduced point source of infection. Ground covers were bare soil, black plastic, or a cover crop of vetch that was grown on raised beds in the fall, killed in the spring and left on the soil surface. At all assessment times, there was a significant reduction in disease severity with increasing distance from the infection source. Severity was significantly lower at most of the distances evaluated in plots with vetch compared with bare soil or plastic. Percentage of diseased and sunburned fruit was 47% lower in plots with vetch than with bare soil or plastic. Soil splash dispersal also was reduced in plots with vetch or plastic versus bare soil. These results indicate that the use of no-till vetch cover crop can reduce the dispersal of spores by rain splash and thus have a significant suppressive impact on the spread of anthracnose on watermelon.

Screening of bacterial antagonists for suppression of sheath blight in rice

X. ZHOU (1), K. Kumar (2), M. Reddy (2), J. Kloepper (2), S. Zhang (3)
(1) AgriLife Research, Texas A&M University System, Beaumont, TX, U.S.A.; (2) Dept. Entomology and Plant Pathology, Auburn University, Auburn, AL, U.S.A.; (3) Tropical REC, University of Florida, Homestead, FL, U.S.A.
Phytopathology 100:S146

Sheath blight caused by *Rhizoctonia solani* is the most important disease of rice in the southern United States. Since none of the leading high-yielding cultivars have acceptable levels of resistance, sheath blight management has been largely depended on fungicides. However, the use of fungicides is costly and unsustainable. Biological control has been recently considered as a promising option. *In vitro* and greenhouse assays were conducted to screen biocontrol agents for suppression of sheath blight in rice. Nineteen bacterial strains that were previously demonstrated growth promotion in other plants and antibiosis against other plant pathogens were examined for their antifungal activity on mycelial growth of *R. solani*, germination of sclerotia and hyphal growth of germinated sclerotia. They were also examined for their

ability to inhibit lesion development on leaf blades and sheaths of seedlings in the greenhouse. Ten out of 70 strains showed significant inhibition of mycelia growth and the hyphal growth of germinated sclerotia. Four of these 10 strains also significantly inhibited the germination of sclerotia. When tested in the greenhouse, 10 strains significantly reduced the lesions on leaf blades and sheaths. The performance of these strains, most of which belong to *Bacillus subtilis*, under field conditions will be further evaluated.

A cucumber mosaic virus mutant that induces resistance to its aphid vector in tobacco

H. ZIEBELL (1), A. Murphy (2), M. G. Lewsey (2), J. H. Westwood (2), K. L. Perry (1), M. Stevens (3), J. P. Carr (2)
(1) Cornell University, Ithaca, NY, U.S.A.; (2) Cambridge University, Cambridge, UNITED KINGDOM; (3) Broom's Barn Applied Crop Sciences, Bury St Edmunds, UNITED KINGDOM
Phytopathology 100:S147

Cucumber mosaic virus (CMV) is transmitted by aphids in a non-persistent manner. The CMV coat protein is the only known factor required for aphid transmission. We have found that the CMV 2b protein plays an indirect but important role in transmission by protecting the vector against the induction of anti-insect defenses. The 2b protein is a counter-defense factor that suppresses the initiation of RNA-silencing, amongst others. While investigating the potential of CMVΔ2b (a CMV mutant in which the gene for the 2b protein is deleted) as a cross-protection agent, we noted that aphid (*Myzus persicae*) infestation was inhibited on CMVΔ2b-infected plants of tobacco (*Nicotiana tabacum*). We found statistically significant decrease in aphid survival and an increases in aphid mortality on CMVΔ2b-infected plants compared to mock-inoculated plants or plants infected with wild-type CMV. The data indicates that in CMVΔ2b-infected plants a viral gene product other than 2b (or the stress of viral infection) induces resistance to aphids. However, in plants infected with wild-type CMV we suspect that this effect is neutralized by the 2b protein. The results suggest that an RNA-silencing suppressor may also target anti-aphid defenses as well as anti-viral responses in plants. Gene expression analysis of jasmonic acid-regulated genes in tobacco support this hypothesis. Furthermore, aphid transmission of CMVΔ2b is drastically reduced compared to wild-type CMV. Work funded by grants from BBSRC and Leverhulme Trust.

Sphingoid bases and their 1-phosphates, but not fumonisins, are translocated from roots to aerial tissues of maize seedlings watered with fumonisins

N. C. ZITOMER (1), S. Jones (2), C. Bacon (1), A. E. Glenn (1), T. Baldwin (1), R. T. Riley (1)
(1) USDA – ARS, Toxicology and Mycotoxin Research Unit, Athens, GA, U.S.A.; (2) South Carolina State University, Department of Biological Sciences, Orangeburg, SC, U.S.A.
Phytopathology 100:S147

In an earlier study using maize seedlings grown from kernels inoculated with *Fusarium verticillioides*, fumonisin B_1 (FB_1) was preferentially accumulated in leaf tissue compared to FB_2 and FB_3. The present study tested whether maize seedlings preferentially translocate FB_1 when plants are watered with FB_1 and/or FB_2, without the fungus present. The results show that neither FB_1 nor FB_2 was translocated when administered in the watering solution and while both FB_1 and FB_2 were taken up by the roots the accumulation of FB_2 in roots was significantly less than predicted indicating that FB_1 was preferentially accumulated. In addition there was clear evidence of ceramide synthase inhibition in the roots and sphingoid base and sphingoid base 1-phosphates accumulated in leaf tissue presumably due to translocation from the roots. These findings suggest that the fungal/plant interaction is necessary for FB_1 translocation in maize seedlings infected with *F. verticillioides*.

Combination of genetic resistance, reduced-risk fungicides and Tom-Cast for tomato disease control

T. A. ZITTER (1), J. L. Drennan (1), M. A. Mutschler (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S147

Late blight (LB), early blight (EB), and Septoria leaf spot (SLS) are the major foliar tomato diseases in temperate regions. Growers currently rely upon fungicides applied on a weekly basis for control. Hybrid tomato varieties with EB tolerance and/or LB resistance are becoming available commercially, and the addition of SLS resistance is progressing. The objectives of this research were: to confirm the need for homozygous resistance for EB, to determine the efficacy of reduced-risk fungicides (azoxystrobin + difenoconazole and boscalid) following Tom-Cast, compared with weekly application of chlorothalonil or an organic practice (*Bacillus subtilis* + cupric hydroxide) for EB and SLS, and to reduce the environmental impact quotient (EIQ) for the fungicides chosen. EB homozygous tolerant genotypes performed significantly better than heterozygous genotypes or susceptible controls over a 3 year period. Genotypes with LBR conferred by *Ph3* plus *Ph2* genes were immune to LB-US22 in 2009. All fungicide treatments provided control of both EB and SLS compared with the unsprayed control. Chlorothalonil when applied weekly developed tolerance to EB, but provided season-long control of SLS. The reduced-risk treatments provided superior control of both diseases with 4 fewer sprays for EB and 3 fewer sprays for SLS control. The EIQ values of these fungicides were 80% lower than those for the conventional or organic treatments.

Silencing of Cysteine protease, acidic chitinase or PR1-a individually, does not hamper BTH mediated resistance to *P. infestans* in tomato

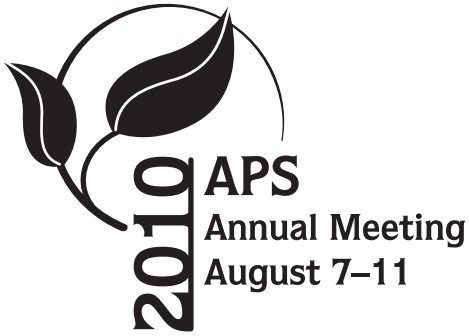
A. ZULUAGA (1)
(1) Cornell, Ithaca, NY, U.S.A.
Phytopathology 100:S147

Induced resistance by chemicals such as benzothiadiazole BTH (Syngenta Inc) mimics the biological activation of Systemic Acquired Resistance (SAR) by necrogenic pathogens. BTH takes the place of salicylic acid (SA) in the SAR signal pathway, inducing the same molecular markers and range of resistance. Previous work in our laboratory found that BTH activates resistance against late blight caused by P. infestans, on petunias and tomatoes while it did not activate resistance against the same pathogen on potatoes, suggesting that the spectra of resistances activated by BTH are crop and pathogen specific. The goal of our work was to understand the molecular mechanism by which BTH mimics the SAR response and further understanding why BTH works in some plants and not others. To address this question we used microarray technology to identify the genes expressed in response to BTH in tomatoes. Of these we selected three candidate genes (cysteine protease, acidic chitinase and PR1-a) to characterize further by silencing using Virus Induced Gene Silencing (VIGS). Our hypothesis was that silencing of these genes will reduce the resistance response observed in plants after BTH treatment. However, silencing of cysteine protease, PR1-a or acidic chitinase II individually did not reduce the effect of BTH on plants. The lack of phenotype after silencing PR1-a supports previous conclusions from our lab that partial resistance to P. infestans in tomato is not dependent of the SA pathway.

Sensitivity of *Fusarium graminearum* isolates to pyraclostrobin

M. ZWINGMAN (1), J. Hernandez Nopsa (1), K. Eskridge (1), S. Wegulo (1)
(1) University of Nebraska, Lincoln, NE, U.S.A.
Phytopathology 100:S147

Fusarium graminearum causes Fusarium head blight (FHB) of wheat as well as ear and stalk rots of corn. Pyraclostrobin is one of the most frequently applied fungicides for control of foliar diseases of wheat and corn in Nebraska. The objective of this study was to determine if Nebraska isolates of *F. graminearum* differed in sensitivity to pyraclostrobin. The isolates were collected from wheat fields and elevators in 2007 following severe epidemics of FHB in the south central and eastern parts of the state. Potato dextrose agar was amended with salicylhydroxamic acid (SHAM, dissolved in methanol) at 100 µg/ml of PDA, then with technical grade pyraclostrobin (95 percent) dissolved in acetone at 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/ml. A 5-mm-diameter PDA mycelial plug from an actively growing edge of each of 15 *F. graminearum* isolates was placed, mycelial face down, at the center of the amended PDA plates which were then incubated at 25°C in 12 hr light and 12 hr dark. An alpha lattice randomized design with 3 replications was used. EC_{50} values calculated from mycelial area measured after 10 days ranged from 0.063 µg/ml to 1.585 µg/ml for 12 isolates. However, isolates NE90, NE101, and NE91 had EC_{50} values of 15.85, 100.0, and 398.1 µg/ml, respectively. These preliminary results indicate the development of resistance to pyraclostrobin in some Nebraska populations of *F. graminearum*.



2010 APS Annual Meeting
Abstracts of Special Session Presentations

Biology of Plant Pathogens

10th I. E. Melhus Graduate Student Symposium: Seed Pathology—Epidemiology, Management, and Phytosanitary Concerns

Quorum sensing affects virulence and seed-to-seedling transmission of *Acidovorax avenae* subsp. *citrulli*, the causal agent of bacterial fruit blotch
K. JOHNSON (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S148

Watermelon fruit and seed production can be significantly limited by bacterial fruit blotch (BFB) caused by *Acidovorax avenae* subsp. *citrulli* (AAC). BFB can cause up to 100% loss in fields and infested seed is the most important source of inoculum. During AAC colonization of seed there is a switch from non-pathogenic to pathogenic growth that could be regulated by quorum sensing (QS). QS is the ability of bacteria to communicate with each other and respond collectively to environmental cues. AAC has the QS homologs, *luxI* and *luxR*, based on genomic sequence analysis. The role of QS in seed colonization and seed-to-seedling transmission was investigated using *luxR* and *luxI* mutants of AAC strain 00-1. The *luxR* and *luxI* mutants were able to colonize germinating watermelon seed to levels similar to wildtype. The QS mutants were efficiently transmitted from seed-to-seedling when high levels of initial inoculum, (10^6 CFU), were used. Seed infiltrated with AAC00-1, the *luxI* or *luxR* mutant had 97.5, 93 and 95% seed-to-seedling transmission, respectively. However, the *luxI* mutant was significantly reduced in its ability to be transmitted from seed-to-seedlings compared to wildtype when low levels of initial inoculum, (10^3 CFU), were used. Seed infiltrated with AAC00-1 or the *luxI* mutant had 76 and 33% seed-to-seedling transmission, respectively. These results suggest that QS plays a role in seed-to-seedling transmission of the BFB pathogen.

Effect of the mechanism of infestation on the localization of *A. avenae* subsp. *citrulli* in naturally infested watermelon seeds
B. DUTTA (1)
(1) University of Georgia, Athens, GA, U.S.A.
Phytopathology 100:S148

Previously, it was determined that watermelon seeds could become infested by *Acidovorax avenae* subsp. *citrulli* (Aac) via two mechanisms: 1) penetration of the pericarp of the ovary that results in fruits with bacterial fruit blotch (BFB) symptoms and 2) invasion of the pistil that results in infested seeds within symptomless fruits. In this study, we investigated the effect of the mechanism of seed infestation on localization of Aac in seeds. Watermelon seeds from symptomatic fruit (pericarp invasion) and asymptomatic fruit (pistil invasion) were tested for Aac by PCR and plating on semi-selective media. Samples (n = 50 seeds) from each type of seedlot were dissected into sections including seed coat (testa), perisperm-endosperm layer (a thin suberized envelope that encloses the cotyledon), and endosperm. The mean proportions of Aac-positive PE layer sections by real-time PCR were not significantly different for pistil (83%) and pericarp-invaded (99%) seedlots. In contrast, for the same seedlots, the mean proportions of Aac-positive endosperm tissue samples were significantly higher for pistil-invaded (98%) than for pericarp-invaded (11%) seedlots. Additionally, less than 8% of the testa samples were Aac-positive for both seedlot types. These results indicate that for seeds infested by pericarp penetration, Aac becomes localized on or outside the PE layer. In contrast, when seeds become invaded by pistil penetration, Aac becomes localized in the endosperm under the PE layer.

Characterization of genes in *Fusarium verticillioides* regulating colonization of maize kernels
R. L. HIRSCH (1), B. H. Bluhm (1)
(1) University of Arkansas, Fayetteville, Fayetteville, AR, U.S.A.
Phytopathology 100:S148

Fusarium verticillioides is a common seedborne pathogen of maize and produces fumonisin mycotoxins during kernel colonization. Identifying genes underlying seed infection is crucial to elucidate pathogenesis at the molecular level. The objective of this research was to characterize genes in *F. verticillioides* that regulate kernel colonization. The overall approach was to identify genes through forward genetics and determine their function through targeted disruption. First, a collection of >3000 random insertional mutants was generated via Restriction Enzyme Mediated Integration (REMI) with a novel promoter-trapping cassette. A high throughput *in vitro* screen was developed to quantify the hydrolysis of starch, the predominant carbohydrate in maize kernels. Nine mutants with altered starch hydrolysis were analyzed for their ability to colonize maize kernels; of these, one mutant was significantly impaired in kernel colonization and fumonisin production. In this mutant, the REMI cassette disrupted a gene encoding a putative ubiquitin ligase (designated *UBL1*). Targeted disruption of *UBL1* in the wild-type strain confirmed the phenotype of the REMI mutant. The discovery of a novel regulatory gene underlying seed colonization and fumonisin biosynthesis significantly expands the working model of kernel pathogenesis in *F. verticillioides.*

Interactions between viruses and *Phomopsis* infection in soybean, and effects of integrated management strategies
J. SOTO-ARIAS (1), G. Munkvold (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S148

Bean pod mottle virus, *Soybean mosaic virus*, bean leaf beetles, soybean aphids and *Phomopsis* spp. all affect soybean seed quality in addition to causing yield losses. However, interactions among these pests and pathogens, and the effects of combined management practices, are not well understood. To understand these interactions, greenhouse studies were established to determine the effects of virus infection on susceptibility of soybean plants to infection by *Phomopsis longicolla* at different growth stages. Virus inoculation with either SMV or BPMV, significantly increased seed infection by *P. longicolla*, compared with the control or plants only inoculated with *P. longicolla*. To evaluate the effects of management strategies, four experiments were established in 6 locations in Iowa during 2008 and 2009. Applications of Headline at R5, or Stratego Pro by itself or in combination with insecticide at

R3, significantly reduced *Phomopsis* spp. infection of stems and seeds. Insecticide applications alone reduced aphid and bean leaf beetle populations. In some experiments insecticide applications also reduced *Phomopsis* spp., SMV and BPMV infection of seeds, but this effect was not consistent. Virus incidence and beetle populations were very low in both years, and seed mottling was not observed. *Phomopsis* spp. infection affected seed germination in some experiments. Few treatments aimed at insect and disease control had an effect on yield.

Advances in Plant Virus Evolution

The evolution of plant virus evolution: A historical overview
W. L. SCHNEIDER (1)
(1) USDA-ARS FDWSRU, Fort Detrick, MD, U.S.A.
Phytopathology 100:S149

Plant viruses face challenges at every phase of their life cycle. There is strong selection pressure to interact with the host during uncoating, translation, replication, cell to cell movement and long distance movement. At the same time there is a constant battle to avoid the host defenses and RNA silencing. In addition, plant viruses face selection pressures during horizontal transmission. From the early days of plant virus research virologists noted the flexible traits of their subjects. Not surprisingly, viruses, with high mutation frequencies and large populations are adept at evolving to deal with new selection pressures and challenges. However, recent research has provided valuable insight into just how these variable populations of RNA viruses, DNA viruses and viroids contribute to plant virus evolution. This symposium covers some of the latest work on plant virus evolution, including population processes, the factors affecting viral emergence, viral breakdown of host resistance, the evolution of plant DNA viruses, and viroid evolution.

Population processes and plant virus evolution
R. FRENCH (1)
(1) USDA, ARS, University of Nebraska, Lincoln, NE, U.S.A.
Phytopathology 100:S149

The number of studies detailing levels of sequence diversity within plant virus populations are growing at a rapid pace. At the same time, recent work has provided empirical estimates of parameters important in the life cycle of plant viruses, which in turn can help in understanding observed patterns of polymorphism. Despite the fact that plant viruses are prolific replicators, producing upwards of millions of virions per cell, they are subjected to severe genetic bottlenecks at virtually all stages of growth, including cell to cell movement, systemic infection, and horizontal transmission to new hosts. Thus, the effective population size (Ne) of plant viruses is many orders of magnitude smaller than their census numbers. Ne is of crucial importance in determining both the rate of genetic drift in a population (drift is faster in smaller populations), and the efficacy of selection relative to drift. Evidence also suggests that intracellular replication of RNA viruses (as well as DNA viruses replicating by a rolling circle mechanism) is a nearly linear 'stamping machine' process. This profoundly reduces the number of mutant genomes that are produced in a viral population. A 'stamping machine' mode of replication also increases the variation in offspring number among potential parental genomes and reduces Ne. Nevertheless, strong selection can still effect changes even in small populations, with better adapted genotypes replacing those with deleterious mutations.

Evolutionary and systems biology of plant RNA virus emergence
S. F. ELENA (1)
(1) Instituto de Biología Molecular y Celular de Plantas, Valencia, SPAIN
Phytopathology 100:S149

Understanding the underlying mechanisms by which viruses are able to overcome the host's defenses and proliferate has been a challenging problem because the large number of cellular factors involved and the complexity of interactions established during infection. The classic approach has been the identification of one or few host genes involved in the interaction. The generalization of the "omic" techniques is opening the possibility of taking a whole picture of the interaction. Here, I present results from an evolution experiment in which Tobacco etch virus has been adapted to *Arabidopsis thaliana*. I show that adaptation has profound effects in the way virus and plant interact. Next, I present a comparative study of the lists of over/under-expressed genes from infection experiments with the potyviruses Tobacco etch virus, Turnip mosaic virus and Plum pox virus, and the phylogenetically unrelated Turnip crinkle virus. We analyze lists in terms of biological functions. Then, taking advantage of the recently inferred regulatory networks of *A. thaliana*, we dissect the viral mode of action showing a directed mechanism by altering the expression of key genes on the interactome. The set of genes specifically responding for phylogenetically related viruses represents interactions that acquired during the evolutionary diversification of a viral family. Those interactions shared by phylogenetically unrelated viruses represent anon-specific responses.

Evolution of natural populations of BNYVV to overcome host resistance
C. M. Rush (1), R. ACOSTA-LEAL (1)
(1) Texas AgriLife Research, Amarillo, TX, U.S.A.
Phytopathology 100:S149

Beet necrotic yellow vein virus (BNYVV), vectored by *Polymyxa betae*, causes rhizomania, a devastating root disease of sugar beet. Genetic resistance against BNYVV, conferred by the single dominant gene *Rz1*, is commercially available in regionally adapted cultivars in all production regions of the U.S.A. In the last few years, cultivars with a second resistance gene, *Rz2*, and cultivars with a combination of *Rz1* and *Rz2* have been released. Based on results of variety trials, it appears that resistance in cultivars with *Rz1 + Rz2> Rz2> Rz1.* BNYVV can be isolated from all of these resistant cultivars but the virus is typically low titer and disease symptoms are absent or minimal. However, in 2002, sugar beets with *Rz1* resistance from the Imperial Valley of California, displayed severe symptoms of rhizomania. Although isolates of BNYVV typically are highly conserved, most isolates from symptomatic *Rz1* plants exhibited an unique aa motif in the hyper variable region of p25 on RNA3. Furthermore, isolates of BNYVV from infected, but asymptomatic, *Rz1* plants were also different from wild type BNYVV. Resistance breaking isolates from other regions of the U.S.A. are similar, but not identical, to California RB isolates. Greenhouse experiments revealed that strength of genetic resistance in the host significantly affects virus mutation frequency.

How do Geminiviruses evolve as quickly as RNA viruses?
S. DUFFY (1)
(1) Rutgers, The State University of New Jersey, New Brunswick, NJ, U.S.A.
Phytopathology 100:S149

Geminiviruses are significant emerging pathogens of crops worldwide. Their ability to emerge and adapt to novel hosts has long been attributed to their frequent recombination. However, evidence is mounting that they evolve quickly in the absence of recombination, and have similar nucleotide substitution rates as RNA viruses of plants and animals. The mechanisms by which geminiviruses could accumulate mutations as quickly as RNA viruses are evaluated: high mutation rates, short generation times and recurrent selective sweeps. Mutation frequencies and mutational spectra suggest that geminiviruses have mutation rates much higher than previously assumed.

Advances in the understanding of viroid evolution
R. W. HAMMOND (1)
(1) USDA, ARS, PSI, MPPL, Beltsville, MD, U.S.A.
Phytopathology 100:S149

Viroids are the smallest known pathogenic agents of plants and cause diseases of considerable economic importance. Their genomes are composed of a single-stranded, covalently-closed, circular, highly-structured RNA molecule of 246 – 401 nt. They are classified into two families—those that replicate in the nucleus (pospiviroids) and those that replicate in the chloroplast (avsunviroids). Viroids lack the capacity to code for proteins, are not encapsidated, and are replicated by host-encoded polymerases. As such, viroids interact with their host through specific structural/sequence motifs for replication, movement, and pathogenesis. Viroid infections are typically characterized by the presence of a population of sequence variants that conform to a quasispecies model and where predominant forms accumulate during infection. Point mutations and RNA recombination contribute to the sequence diversity of viroids, and the requirements to maintain conserved structures, the host response to infection, and environmental selective pressures all contribute to influence the population of variants. Analysis of accumulated sequence data from natural and experimental populations of pospi- and avsunviroids has led to the proposal of several intriguing models of viroid RNA evolution. In addition to a discussion of these models, the implications of viroid evolution to agriculture will be discussed.

Refining Systematics (Taxonomy, Nomenclature, Phylogenetics) for Better Resolution in the Population Biology and Evolution of the Oomycetes

Pythium, _Pythiogeton_ and prov. name _Phytopythium_: The current status for the species in the genera

A. W. DE COCK (1), G. P. Robideau (2), K. Bala (2), M. D. Coffey (3), Z. G. Abad (4), C. A. Lévesque (2)
(1) CBS-KNAW Fungal Biodiversity Centre, Utrecht, NETHERLANDS; (2) Agriculture and Agri-Food Canada, Ottawa, CANADA; (3) Department of Plant Pathology and Microbiology, University of California, Riverside, CA, U.S.A.; (4) USDA-APHIS-PPQ-PHP-RIPPS-Molecular Diagnostics Laboratory, Beltsville, MD, U.S.A.
Phytopathology 100:S150

Traditionally, genera and species in Oomycetes have been distinguished and defined based on morphological characteristics. The genus *Pythium* is characterized by its well developed hyaline mycelial thallus and the way zoospores are developed and discharged: the sporangium forms a discharge tube through which the contents move out and form a vesicle at the tip with an undifferentiated mass of protoplasm. This mass then differentiates into biflagellate zoospores. Although this way of zoospore discharge is shared by all *Pythium* species, the genus is heterogeneous with regard to morphological characters like e.g. the sporangium shape. The genera *Pythiogeton* and *Lagenidium* display a way of zoospore discharge similar to that in *Pythium*, though they are considered different genera based on other characteristics. DNA sequence analyses allow an evaluation of the morphological classification over a phylogenetic framework. Analysis of ribosomal DNA regions and the mitochondrial COI showed that a clade within *Pythium* is actually more closely related to *Phytophthora* than to *Pythium*; this clade is provisionally named *Phytopythium*. Moreover, molecular analyses revealed the close relationship of *Pythiogeton* and some *Lagenidium* species to *Pythium*. The phylogenetic results will be discussed with regard to morphology.

How to avoid misidentifying your isolates: The value of the Morphological / Phylogenetic Key of _Phytophthora_ extypes and neotypes

Z. ABAD (1)
(1) USDA-APHIS-Molecular Diagnostics Laboratory, Beltsville, MD, U.S.A.
Phytopathology 100:S150

Phytophthora with 105 species is a major genus of plant pathogens. Although there have been considerable advances in its molecular taxonomy, there is still confusion in recognizing new *Phytophthora* species and in identifying described species. This confusion is due in part to the great number of misidentified or incorrectly annotated sequences submitted to the GenBank. Such errors in identification make it difficult to recognize many of the clusters of the "sensu stricto". The "Holotype" (= Type) is the single isolate that defines the species and the "Ex-holotype" is the isolate originated from the "Holotype". Interestingly, taxonomic manuscripts published after the description of *P. infestans* in 1876 (until present) rarely contain information on the codes of the types. This information, which has rarely been presented, is vital for refining the systematics of the Genus. We are reviewing the original manuscripts to compile information of the Primary Types, assigning Lectotypes and selecting potential Neotypes. Our goal is to establish a database of sequences of the types and to use selected cultures to develop a Morphological/Phylogenetic Phytophthora Key, and to publish a manuscript to update the Taxonomy of the Genus. The USDA-APHIS-MDL is collaborating with the World Phytophthora Collection and the Phytophthora Database on this important initiative. We expect that the database and the key will be useful tools to avoid misidentifying isolates of this important genus.

The _Phytophthora_ Database: Current status and future directions

S. KANG (1)
(1) Department of Plant Pathology, Penn State, University Park, PA, U.S.A.
Phytopathology 100:S150

The *Phytophthora* Database (http://www.phytophthoradb.org) supports rapid identification of *Phytophthora* species via comparison of sequences at one or more marker loci. Besides archiving marker sequences from most of the known and newly discovered species to support identification and new species description, the database provides a comprehensive overview of *Phytophthora* molecular diagnostics, morphological and pathological characteristics of many of the archived species, and references. Data search and analysis tools in the database include BLAST, Phyloviewer (a program for building phylogenetic trees using selected sequences), Virtual RFLP (a program for generating expected restriction patterns for given sequences), GIS tool (a program for visualizing the geographic origins of species and isolates through a global map), and Cart & Folder (a customized means of storing and sharing data via the database). The current status and future directions of the database will be presented.

The Oomycetes Database: The initiative for an international web-based informatics platform

F. N. MARTIN (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 100:S150

Over the years the taxonomic classification of Oomycetes based on morphological features has been in a state of flux and the application of molecular techniques has not always provided the clarity that is desired. In part this has been due to a lack of consistency of the loci that have been sequenced as well as a historical under representation of some groups of organisms among the different studies. The objective of this project is to establish a collaborative initiative among researchers working this diverse group of organisms to facilitate a broader scale analysis of the kingdom using the same set of nuclear and mitochondrially encoded loci. This data will be presented on a web-based informatics platform patterned on what has been developed for *Phytophthora* (www.phytophthoradb.org). In addition to providing sequence data and a comprehensive multigene phylogenetic analysis, there will also be an overview on the biology and ecology of the orders, genus and species descriptions and their morphological features, tools supporting species identification based on molecular and morphological criteria and visualizing the geospatial, environmental, and/or temporal contexts of archived species and isolates. Efforts will initially focus on analysis of the Peronosporomycetidae (*sensu* Dick) and will be expanded at a later time to include members of the Saprolegneomycetidae.

Mitochondrial genomics of Oomycetes, tools for phylogenetics and development of molecular markers

F. N. MARTIN (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 100:S150

Due to its comparatively small size, similar number of genes and rate of evolutionary divergence the mitochondrial genome can be a valuable resource for elucidating phylogenetic relationships and development of molecular makers. In an effort to facilitate the use of this region for these purposes the mitochondrial genomes of 15 *Pythium* and 20 *Phytophthora* spp. have been sequenced. Comparative genomics has been useful for identification of genes useful for estimating evolutionary relationships and development of conserved primer sequences for their amplification. A mitochondrial multigene phylogeny for the genus *Phytophthora* was recently completed and efforts are underway to include *Pythium* and other Oomycetes in the analysis using the same regions. Comparison of genomic sequences among *Phytophthora* spp. has identified the types of polymorphisms associated with intraspecific compared to interspecific genome evolution. This has facilitated the identification of regions more prone to evolutionary divergence that are useful for classification of mitochondrial haplotypes. Conserved mitochondrial gene order differences among *Phytophthora* compared to *Pythium* and plants have also been useful for development of a systematic approach for development of multiplexed TaqMan real time PCR diagnostic marker system for identification of *Phytophthora* at a genus as well as species specific level. A similar approach is under investigation for *Pythium* as well.

Population genetic insights into emergence of oomycete pathogens

N. J. GRUNWALD (1)
(1) USDA ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S150

Oomycetes include notable pathogens that have repeatedly emerged as significant threats to plant biosecurity. Among these are for example the sudden oak death pathogen Phytophthora ramorum and the potato late blight pathogen P. infestans. Population genetic tools, whether based on molecular markers such as microsatellites or nucleic acid sequences, can provide unique insights into the evolutionary dynamics underlying invasion or emergence of Oomycete plant pathogens. Select examples of population genetic approaches used to understand the emergence of Oomycete pathogens will be presented and explored.

Aquatic habitats—A reservoir for population diversity in the genus _Phytophthora_

J. HWANG (1), S. N. Jeffers (1), S. W. Oak (2)
(1) Clemson University, Clemson, SC, U.S.A.; (2) USDA Forest Service, Southern Region-FHP, Asheville, NC, U.S.A.
Phytopathology 100:S150

Occurrences of oak decline and sudden oak death in forests of Europe and the west coast of the U.S.A., respectively, have focused attention on the species of *Phytophthora* present in natural ecosystems. We have been investigating the diversity of species of *Phytophthora* present in forest streams in the eastern U.S.A. *Phytophthora* spp. are well adapted to aquatic environments and can be recovered from stream water by baiting and filtration. Extensive surveys in

multiple states revealed that a diversity of species occurs naturally in forest streams. In one study, five forest streams in western North Carolina were monitored monthly for a year. Seven species—*P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, and *P. pseudosyringae*—and seven morphologically and genetically distinct groups of isolates were detected. Samples of stream-side soils and plants with symptoms also were collected, but only three species were detected: *P. cinnamomi* and *P. heveae* in soils and *P. citricola* and *P. heveae* on plants. Species of *Phytophthora* consistently were detected in streams during winter months when air temperatures were near or below freezing, which are not conducive to lesion development and sporulation. These results suggest that the native population of *Phytophthora* spp. in stream water is different from those in terrestrial habitats. The species of *Phytophthora* present in streams may occupy a unique niche—i.e., they appear to be aquatic inhabitants and not transient visitors.

Ecological adaptations in Phytophthora. Understanding their role in forest ecosystems
Y. BALCI (1)
(1) University of Maryland, College Park, MD, U.S.A.
Phytopathology 100:S151

We have examined two species of *Phytophthora* for their role in plant health in Appalachian oak ecosystems; one from soil and the other from streams; *P. cinnamomi* and *P. appalachiensis*, respectively. *P. cinnamomi* was found to be the most common and widespread species in eastern U.S. oak forests during a multi State survey. *P. appalachiensis* has been identified as a new species from a stream in West Virginia. It could only be isolated during June-October and no other species was isolated from the same stream. It also was found infecting fallen leaves in the stream and live foliage if shoots of rhododendrons were dipped into the stream. During leaf inoculations, it was pathogenic, but significantly more when wounded. *P. cinnamomi* was the most common *Phytophthora* species below the N 40° latitude range. Its occurrence in the eastern U.S. oak forests most likely is restricted by the low minimum temperature extremes as reflected by the overlapping incidences with plant hardiness zone maps. In infested sites, multiple woody plants harbored the pathogen. When examined with the oak decline incidences in Ohio, we found significant root mortalities on infested white oaks (*Q. alba*) and greater inoculum levels in lower moist bottomlands. This pathogen appears to be mainly affecting tree health by killing fine roots particularly when site conditions are favorable, whereas, *P. appalachiensis* seems to be opportunistic in behavior.

The Sophistication of Host-Pathogen Interactions Involving Necrotrophic Fungi

Live and let die: The smart lifestyle of *Botrytis cinerea*
J. A. VAN KAN (1)
(1) Wageningen University, Wageningen, NETHERLANDS
Phytopathology 100:S151

It becomes increasingly apparent that interactions between plants and necrotrophic fungi are surprisingly subtle and complex, and host plants in fact play a much more active role in disease than previously anticipated. Just causing 'death' isn't good enough, the execution of programmed cell death by a host plant in response to a pathogen is crucial for many necrotrophs to be successful. *Botrytis cinerea* is a ubiquitous pre- and post-harvest pathogen infecting a wide range of host plants and tissues. I will present an overview of current knowledge on pathogenicity factors of *B. cinerea*, with emphasis on phytotoxic metabolites and proteins that can cause (programmed?) plant cell death. I will subsequently discuss processes occurring in the host plant during the interaction, with emphasis on the formation of Reactive Oxygen Species and nitric oxide, as well as on cell death pathways. Examples will be presented of host defense responses during *B. cinerea* infection, that contribute to (partial) resistance. The capacity of *B. cinerea* to counteract the growth inhibitory activity of defence compounds, by a combination of enzymatic detoxification and secretion mechanisms, also contributes to its successful lifestyle.

Necrotrophy in *Sclerotinia sclerotiorum*: To oxalate and beyond
J. ROLLINS (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S151

Oxalate production is positively associated with the broad host range macerating symptoms of *Sclerotinia sclerotiorum* diseases. Yet, many non-pathogens produce oxalate; many pathogens manifesting different symptoms produce oxalate; and other *Sclerotinia* species produce oxalate but have unique or restricted host ranges. What attributes of oxalate regulation and what other factors account for the host range and symptomatology of *S. sclerotiorum* disease? Our studies have revealed that the ambient pH environment, via Pac1 molecular regulation, plays a key role in controlling oxalate accumulation. This regulation appears to act directly on at least one structural gene in the oxalate biosynthetic pathway. This gene, *oah1*; encoding oxaloacetate acetylhydrolase, is down-regulated in the low-oxalate *pac1* loss-of-function mutant. Two independently isolated *oah1* deletion mutants fail to accumulate oxalate in culture or *in planta*. Despite the lack of oxalate production, these mutants infect a range of host plants albeit, with greatly attenuated symptoms. In addition, their culture filtrates retain necrosis-inducing activity when infiltrated into host leaves. These findings indicate that factors other than oxalate may be responsible for establishing basic compatibility. Current investigations are aimed at distinguishing between direct toxic roles and more subtle host-modulating activities of oxalate and in identifying other factors that condition host-pathogen compatibility.

Systematic characterization of the kinome of the wheat scab fungus Fusarium graminearum
J. XU (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S151

Wheat scab caused by Fusarium graminearum is one of the most important diseases of wheat. Beside yield losses, infested wheat kernels are often contaminated with mycotoxins. Like in many other eukaryotes, protein kinases play major regulatory roles in filamentous fungi. In F. graminearum, there are 126 predicted protein kinases that belong to different protein kinase groups and families. To determine their functions, we have undertaken a systematic approach to generate gene replacement mutants. For a number of protein kinase genes, we were able to isolate mutant although their orthologues are essential in the budding yeast. All the mutants have been assayed for their defects in wheat head infection, DON production, conidiogenesis, sexual reproduction, responses to various stresses, and hyphal growth. Several protein kinases were found to be important for pathogenesis and conidiogenesis. The interaction among these protein kinases and their association with other F. graminearum proteins were predicted based on their yeast orthologues. For a few predicted pathways or networks that are important for plant infection, affinity purification and co-immunoprecipitation assays will be used to determine their interactions in vivo.

Pathogen hijacking of disease resistance mechanisms in wheat
J. D. FARIS (1), Z. Zhang (2), S. Lu (2), H. Lu (3), J. Fellers (4), S. Cloutier (5), S. Xu (2), R. Oliver (6), J. Rasmussen (2), S. Meinhardt (2), T. Friesen (2)
(1) Northern Crop Science Laboratory, USDA-ARS, Fargo, ND, U.S.A.; (2) Fargo, ND, U.S.A.; (3) Amarillo, TX, U.S.A.; (4) Manhattan, KS, U.S.A.; (5) Winnipeg, Manitoba, CANADA; (6) Murdoch, AUSTRALIA
Phytopathology 100:S151

Plant disease resistance is often conferred by genes with NBS-LRR or protein kinase (PK) domains. Much less is known about mechanisms of susceptibility, particularly to necrotrophic fungal pathogens. The pathogens that cause the diseases tan spot and Stagonopora nodorum blotch on wheat produce effectors (host-selective toxins) that induce susceptibility in wheat lines harboring corresponding toxin sensitivity genes. The effector ToxA is produced by both pathogens, and sensitivity to ToxA is governed by the *Tsn1* gene in wheat. We cloned *Tsn1* and found that it contains features of disease resistance genes, including PK and NBS-LRR domains. Mutagenesis revealed that all three domains are required for ToxA sensitivity, and hence disease susceptibility. *Tsn1* alleles are unique to ToxA-sensitive genotypes and insensitive genotypes are null. Sequencing and phylogenetic analysis indicated that *Tsn1* arose in the B-genome diploid progenitor of polyploid wheat through a genome shuffling event that gave rise to its unique structure. Functional analysis indicated that the Tsn1 protein does not interact directly with ToxA. *Tsn1* transcription is tightly regulated by the circadian clock and light, providing further evidence that *Tsn1*-ToxA interactions are associated with photosynthesis pathways. This work suggests that these necrotrophic pathogens thrive by subverting the resistance mechanisms acquired by plants to combat other pathogens.

Dissection of effector-induced host susceptibility pathways in *Stagonospora nodorum* blotch of wheat
S. LU (1), T. L. Friesen (1), J. D. Faris (1)
(1) USDA-ARS Northern Crop Science Laboratory, Fargo, ND, U.S.A.
Phytopathology 100:S151

The necrotrophic *Stagonospora nodorum*-wheat interaction is characterized by several pathogen-derived proteinaceous host-selective toxins (SnToxA, SnTox1, SnTox2, SnTox3 and SnTox4) that induce diseases in the host carrying a corresponding dominant susceptibility gene (*Tsn1*, *Snn1*, *Snn2*,

Snn3 and *Snn4*, respectively). The major susceptibility gene *Tsn1* has been found to encode a novel protein kinase-NBS-LRR disease resistance-like protein (Faris et al, unpublished) that does not directly interact with SnToxA. To dissect the pathways associated with these toxin-susceptibility gene interactions, we have undertaken yeast two-hybrid studies in conjunction with co-immunoprecipitation to identify wheat proteins that are directly targeted by the toxin or interact with the host susceptibility gene product. Several new ToxA-interacting proteins were identified including members of the pathogenesis-related protein (PR) families. Preliminary cDNA library screening also revealed that Tsn1 may interact with a protein potentially involved in the transfer of lipid receptors to the plasma membrane and two chloroplast proteins known to be involved in photosynthesis. These raise the possibility that Tsn1 may have a dual function and likely act as a key mediator for ToxA internalization. Results from further characterization of these candidate Tsn1-interacting proteins will be presented. Hypotheses on how *Tsn1* governs ToxA-induced susceptibility in wheat will be discussed.

Diseases of Plants

Biology and Management of *Rhizoctonia* Diseases in Turfgrass Systems

New *Rhizoctonia*-like pathogens associated with diseases of warm-season turfgrasses
P. F. HARMON (1), S. Kammerer (1)
(1) University of Florida, Gainesville, FL, U.S.A.
Phytopathology 100:S152

Fungi with *Rhizoctonia*-like biology recently have been associated with a range of disease symptoms on warm-season turfgrasses in the Southeast. These fungi lack fruiting bodies, have hyphae of regular diameter with right-angle branching, and occasionally form sclerotia of various size and color on agar media. Disease symptoms range from discrete patches or rings of blighted turfgrass to diffuse canopy thinning and foliar necrosis. Timing of symptoms typically occurs during the hottest months of a year, but symptoms may become more noticeable in fall or periods of semi-dormancy. Recovery has been observed to take an extended period of time despite extensive fungicide application and even after environmental factors begin to favor turfgrass growth and recovery. Turfgrass hosts associated with these symptoms include all warm-season species suitable for amenity uses such as cultivars of bermudagrass and seashore paspalum. Isolates are not always easily obtained from symptomatic turfgrass, especially after the initial symptom development, but plating on a medium amended with a benzimidazole fungicide increases odds of isolation. Phylogenetic analysis of informative sequences indicates isolates form distinct clades related to the *zea*, *oryzae*, and *circinata* varieties of *Waitea circinata*. Additional work is needed to determine if these fungi constitute new varieties of *W. circinata* or if the anamorphic diversity and lack of known teleomorphs warrant designation of new *Chrysorhiza* spp.

The biology of brown ring patch disease on cool-season turfgrasses
F. P. WONG (1), C. Chen (1), K. A. de la Cerda (1), G. W. Douhan (1), L. Stowell (2)
(1) University of California-Riverside, Riverside, CA, U.S.A.; (2) PACE Turf LLC, San Diego, CA, U.S.A.
Phytopathology 100:S152

Brown ring patch is an emergent disease of cool season turfgrass in the U.S. caused by a variety of *Waitea circinata* [proposed as var. *circinata* (Wcc)]. The pathogen was described as causing 'brown ring patch' on creeping bentgrass (*Agrostis stolonifera*) in Japan in 2005. Following outbreaks of yellow rings associated with a Rhizoctonia-like pathogen on annual bluegrass (*Poa annua*) in the western U.S. in 2003, Wcc was indentified as the causal agent of the disease. The disease was subsequently diagnosed throughout the U.S. on annual bluegrass, on roughstalk bluegrass (*P. trivialis*) in the southeastern and southwestern U.S., and a few locations in the western U.S. on creeping bentgrass. Characterization of a diverse collection of Wcc using the ribosomal intergenic spacer and beta tubulin sequences differentiate it from other *W. circinata* anamorphs (*Rhizoctonia* oryzae and *R. zeae*) and from Thanatephorous and Ceratobasidium species. Experiments have demonstrated that Wcc is genotypically diverse, insensitive to benzimidazole fungicides, but can be controlled by other Rhizoctonia-active fungicides, especially flutolanil, polyoxin-D and certain demethylation inhibitors (DMIs). Unlike some Rhizoctonia diseases, brown ring patch is less severe on putting greens with higher nitrogen fertility. Understanding these differences between Waitea, Thanatephorous and Ceratobasidium species is critical for their effective management on turfgrass.

Management of leaf and sheath spot of ultradwarf bermudagrasses
B. MARTIN (1)
(1) Pee Dee Res & Ed Center, Clemson University, Florence, SC, U.S.A.
Phytopathology 100:S152

Leaf and sheath spot, caused by *Rhizoctonia zeae* and *Rhizoctonia oryzae*, have been well documented as pathogens of cool season grasses during warm to hot (28–38 C) and humid weather. Although studies are fewer, isolates of these fungi have also been proven pathogenic to warm-season grasses, including bermudagrass (*Cynodon* spp.), centipedegrass (*Eremochloa ophiuroides*), St. Augustinegrass (*Stenotaphrum secundatum*) and seashore paspalum (*Paspalum vaginatum*). Concurrent with the adoption of 'ultradwarf' bermudagrasses, leaf and sheath spot has increased in locations utilizing these grasses. Identification has been based on cultural characteristics and sequencing of the ITS1 and ITS2 regions of the rDNA. *Rhizoctonia zeae* has been most commonly identified, but *R. oryzae* and *R. circinata* var. *circinata* have also been isolated. Symptoms include rings and patches of a few centimeters up to a meter. Symptoms in transition zone environments may persist for months and fungicides have frequently been unsuccessful in alleviating symptoms as the turf slows in growth. Turfgrass culture in sandy root zones with low fertility has been associated with increased severity, as well as practices that injure the turf during periods of low recovery potential. Management is based on alleviating nutrient stress, avoiding injurious cultural practices, and use of effective fungicides. Preventive applications of azoyxystrobin, pyraclostrobin, fludioxanil, flutolanil, and propiconazole have been shown to be efficacious.

***Rhizoctonia* species causing turfgrass disease in the transition zone: Identification, host resistance and management**
B. J. HORVATH (1), D. S. McCall (2), B. S. Amaradasa (3), M. A. Cutulle (4), V. R. Sykes (1)
(1) University of Tennessee, Knoxville, TN, U.S.A.; (2) Virginia Tech, Blacksburg, VA, U.S.A.; (3) Virginia Tech, Blacksburg, TN, U.S.A.; (4) Virginia Tech, Ellicott City, MD, U.S.A.
Phytopathology 100:S152

Rhizoctonia species cause some of the most destructive diseases on turfgrasses grown in the transition climatic zone. There are currently at least five *Rhizoctonia*-like pathogens that infect cool season turfgrasses in the transition zone. My lab has focused on a three-fold approach to better understanding the pathogen(s) involved in causing disease. First, correct identification of the pathogen is critical for the development of host resistance and disease control. Current work is using various molecular techniques (ITS sequencing, UP-PCR, AFLP, SCAR) to attempt to better understand the relationships within and among the *Rhizoctonia* species causing disease. Second, digital imaging techniques have been developed to accurately assess the potential for host resistance in tall fescue (*Festuca arundinacea*) germplasm accessions. These techniques are also useful to quantitatively assess disease that is present on turfgrass plants. Third, cultural management tools such as, mowing height and N fertility, and chemical management tools such as; nozzle types and granular fungicide applications are being examined to provide new solutions for disease control in the transition zone while attempting to minimize the impact of blanket preventative fungicide applications.

Cryptic Foes: Gathering the Latest Advances on *Pythium*

Ecology and biology of *Pythium* spp. and their impact on crop production
F. N. MARTIN (1)
(1) USDA-ARS, Salinas, CA, U.S.A.
Phytopathology 100:S152

Pythium spp. occupy a diverse ecological habitat ranging from terrestrial ecosystems to aquatic habitats. The genus has a world-wide distribution with over 120 species described, all but a few of which are homothallic. Although some species are not important as pathogens of economic crop plants (some have shown promise as biocontrol agents), a large number of them are responsible for causing diseases ranging from pre- and post emergence damping-off to reduced vigor and yield of mature plants due to root pruning. Some pathogenic species have a broad host range and are capable of attacking

a range of plants while others have a more limited host range or may be restricted more to graminaceous hosts. Differential levels of virulence may also be observed with some species having a significant impact on a particular host while others may be capable of root colonization but cause limited disease. Management of these diseases has relied primarily on fungicides and cultural practices as host resistance is not widely encountered. Other resident microflora can have a profound effect on disease incidence, which can be useful in the development of biological approaches for disease management. Recent efforts to clarify the taxonomic boundaries of species in the genus and evaluate the population biology of some species using molecular tools will facilitate future research on this important genus of plant pathogens.

Sampling and processing of samples for *Pythium*

G. W. MOORMAN (1)
(1) Pennsylvania State University, University Park, PA, U.S.A.
Phytopathology 100:S153

Pythium species are readily isolated from soil, sediment, water, or infected mycelium, roots, stems, or fruits by plating directly on media or media amended with antibiotics. Semi-selective media do not work equally well for all species or against all contaminants. *Pythium* can grow into solid medium that is nutrient-poor and other organisms eliminated by transferring hyphal tips to fresh media. Alternatively, parts of a host plant or other bait can be employed. Each bait is semi-selective in that certain species of *Pythium* readily colonize it while others do not. A protocol that fails to eliminate Pythium in one study may be a good one for *Pythium* work. The rapid colonization of the cut edges of rhododendron leaf disks by *Pythium* reported by *Phytophthora* researchers leads them to use whole leaves but indicates that *Pythium* researchers should use leaf disks. That old seeds produce seedlings highly susceptible to *Pythium* indicates that old seeds or seedlings grown from old seeds could be effective baits or trap plants for *Pythium*. Once in pure culture, there are simple methods of storing Pythium without frequent sub-culturing. Blocks of colonized water agar suspended in sterile tap water at room temperature works well as does inoculating sterile hemp seeds suspended in sterile tap water stored at room temperature. Bacteria greatly shorten the duration of *Pythium* viability in storage. Water agar amended with rose bengal (50–100 µg/ml) is often sufficient to eliminate bacteria.

Assessment of *Pythium* diversity in forest nurseries

J. WEILAND (1)
(1) USDA ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S153

Pythium species are one of the most important and common damping off pathogens affecting conifer seedling production in the Pacific Northwest. Seedling losses can approach 100% when soil moisture is abundant. Despite their prevalence and importance, relatively little is known about the species of *Pythium* found in nursery soils. A limited number of studies report that *P. irregulare*, *P. mamillatum*, and *P. ultimum* are the predominant species in the PNW, but most studies do not report *Pythium* species identity. In an attempt to further characterize *Pythium* species associated with conifer seedling production, a field survey was conducted at three forest nurseries (2 in OR, 1 in WA) in 2008. *Pythium* species were isolated by plating soil onto PARP and by baiting with Rhododendron leaf disks and split Douglasfir needles. One hundred isolates were randomly selected from each method and nursery and identified on the basis of ITS sequence. A total of 19 *Pythium* species were identified from the survey. Species richness and abundance were strongly influenced by both nursery and method. Each nursery was associated with a different predominate *Pythium* species (*P. dissotocum*, *P. irregulare*, and "*P. vipa*").

Role of *Pythium* spp. in the seedling disease complex on cotton; results from the National Cottonseed Treatment Trials

S. A. Winters (1), C. S. ROTHROCK (1), E. E. Gbur (1), L. L. Verhalen (2), T. S. Isakeit (3), W. E. Batson (4), F. M. Bourland (5), P. D. Colyer (6), H. W. Kaufman (7), T. A. Wheeler (8), G. L. Sciumbato (9), K. S. Lawrence (10), A. Y. Chambers (11), P. M. Thaxton (12), W. S. Gazaway (10), T. L. Kirkpatrick (13), P. M. Phipps (14), D. R. Sumner (15), L. J. Littlefield (2), G. B. Padgett (16), F. M. Shokes (14), R. B. Hutmacher (17), R. M. Davis (18), K. W. Seebold (19), J. D. Mueller (20), J. D. Barham (13), M. A. Newman (11), R. H. Garber (21)
(1) University of Arkansas, Fayetteville, AR, U.S.A.; (2) Oklahoma State University, Stillwater, OK, U.S.A.; (3) Texas AgriLife Research, College Station, TX, U.S.A.; (4) Mississippi State University, Mississippi State, MS, U.S.A.; (5) University of Arkansas, Keiser, AR, U.S.A.; (6) LSU AgCenter, Bossier City, LA, U.S.A.; (7) Texas AgriLife Extension, Lubbock, TX, U.S.A.; (8) Texas AgriLife Research, Lubbock, TX, U.S.A.; (9) Mississippi State University, Stoneville, MS, U.S.A.; (10) Auburn University, Auburn, AL, U.S.A.; (11) University of Tennessee, Jackson, TN, U.S.A.; (12) Mississippi State University, Cypress, TX, U.S.A.; (13) University of Arkansas, Hope, AR, U.S.A.; (14) Virginia Tech University, Suffolk, VA, U.S.A.; (15) University of Georgia, Tifton, GA, U.S.A.; (16) LSU AgCenter, Winnsboro, LA, U.S.A.; (17) University of California, Davis, Five Points, CA, U.S.A.; (18) University of California, Davis, Davis, CA, U.S.A.; (19) University of Kentucky, Lexington, KY, U.S.A.; (20) Clemson University, Edisto, SC, U.S.A.; (21) USDA, Shafter, CA, U.S.A.
Phytopathology 100:S153

Fungicides are universally sold on cottonseed to control a number of seed-borne and soilborne pathogens that affect the germination of seed and emergence, survival and vigor of seedlings. The National Cottonseed Treatment Program evaluates seed treatment combinations in 15 to 20 trials annually. The importance of *Pythium* species in the seedling disease complex on cotton in these trials was examined with the fungicide treatment metalaxyl, disease ratings, and pathogen isolations from seedlings and soil populations from the non-treated control plots at each location. Fungicides improved stand over the non-treated control in 120 of the 214 trials conducted by cooperators, 56% of the trials. For the 120 trials with a fungicide response, metalaxyl alone gave a significant response in 40 trials indicating the importance of *Pythium* spp. in these trials. Based on seedling stand response for the metalaxyl treatment compared to seed not treated, responses were found more frequently and stand responses were greater as minimal soil temperatures at planting decreased from 20 to 12 C and total rainfall increased the first three days after planting. Isolation frequency from seedlings and soil populations of *Pythium* spp. were poorly correlated with disease symptoms and stand response suggesting these data have a limited role in characterizing the importance of *Pythium* spp. in the seedling disease complex on cotton.

***Pythium* species associated to plants: The aggressive vs. the moderately, low and non aggressive**

Z. ABAD (1)
(1) USDA-APHIS-Molecular Diagnostics Laboratory, Beltsville, MD, U.S.A.
Phytopathology 100:S153

The genus *Pythium*, with over 150 known species is an exceptional group of organisms not only pathologically, but also ecologically and physiologically. *Pythium* species occupy a high level of niche diversity in aquatic and terrestrial environments world wide that is not observed in other like-fungi or fungi. Plant pathogenic species present levels of pathogenicity including: high, moderate, low and non-pathogenic. High and moderate pathogenic species comprise only the 25% of the reported species, but they can have devastating impact on crops of economic importance around the world. Although numerous species are known to be major plant pathogens, they are frequently omitted in disease diagnosis or considered to be secondary pathogens. Identification of *Pythium* isolates often stop at the Genus level. Some pathogenic species have wide host ranges and are widely distributed (i.e. *P. aphanidermatum*), others are host and locality specific (i.e. *P. solare*). The wide ranges for highly and moderately pathogenic species is very ample but turfgrasses and other related hosts (corn, wheat, oats, barley, rice, and sugarcane) are highly susceptible to *Pythium* and a great number of species have been found associated to these hosts. Factors that influence in the pathogenicity of species will be evaluated as well as the correct morphological and molecular characterization of species which is important in order to apply the adequate measures for the control of the Pythium diseases.

DNA barcode, genomics and phylogenetics of *Pythium* species

C. LEVESQUE (1), G. P. Robideau (2), A. W. de Cock (3), K. Bala (1), Q. A. Eggertson (2), T. L. Rintoul (1), J. Hamilton (4), N. Tisserat (5), C. Buell (4)
(1) Agriculture and Agri-Food Canada, Ottawa, ON, CANADA; (2) Department of Biology, Carleton University, Ottawa, ON, CANADA; (3) CBS KNAW Fungal Biodiversity Centre, Utrecht, NETHERLANDS; (4) Department of Plant Biology, Michigan State University, East Lansing, MI, U.S.A.; (5) Colorado State University, Ft. Collins, CO, U.S.A.
Phytopathology 100:S153

High throughput Sanger sequencing has changed the way we do taxonomy and next generation sequencing is changing the way we do genomics. The goal of DNA barcoding is to generate an identification reference database by sequencing one or a few genes for all species. Some significant advances were made in *Pythium* by the sequencing of both ITS and cytochrome oxidase I (COI) for approximately 1000 strains covering all known *Pythium* species. Both of these markers provide an appropriate amount of resolution and show good complementarity. Several species that were conspecific with ITS remained as such with COI but *P. graminicola*, *P. periilum*, and *P. tardicrescens* were among the notable exceptions of significance to plant pathology. The sequence of the genome of *P. ultimum* var. *ultimum* was recently completed, showing unique features of this plant pathogen compared to *Phytophthora*. A second strain of *P. ultimum* was sequenced and comparative analyses were performed to find highly variable genes. The *P. ultimum* complex with the two different varieties was analysed using these highly variable genes to test the phylogenetic species concept within this group. Additional genomes are being sequenced in other *Pythium* clades using

next generation sequencing. This will reveal more on the pathogenicity mechanisms of the genus and give better tools to resolve the species complexes within *Pythium*.

Population genetics and inter-species boundaries within the *Pythium irregulare* complex
C. D. GARZON (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S154

The genus *Pythium* Pringsh. includes more than a hundred species. Historically described on the basis of few morphological characters, the validity of many species and the systematics of the genus have been questioned. With the advent of molecular phylogenetic tools, several attempts have been made to revise the systematics of *Pythium*. Recent phylogenetic and population genetics studies of several morphologically defined *Pythium* species have revealed the existence of multiple cryptic and closely related species within species complexes. *Pythium irregulare sensu lato* is a morphologically diverse group of species within the F clade of *Pythium* that also has highly polymorphic molecular markers. Previous studies based on ribosomal DNA sequences have identified four highly divergent lineages within *P. irregulare*, including some newly described species. Analyses of multiple genomic and mitochondrial DNA sequences suggest more species should be described. Resolving closely related species in this complex is challenging since most are impossible to distinguish morphologically and their gene sequences can be highly similar. The reported research uses multigene phylogenies to define inter-species boundaries within the *P. irregulare* complex complemented with population genetic analyses to determine the levels of genetic exchange between the putative sister species. These approaches support the occurrence of genetically isolated, cryptic species within the *P. irregulare* complex.

Virus Fishing with Chips: Plant Virus Microarrays and Next Generation Sequencing

Universal plant virus microarray development and validation
B. Bagewadi (1), K. Fischer (2), D. C. Henderson (3), R. L. Jordan (3), D. Wang (4), K. L. Perry (5), U. Melcher (6), J. Hammond (3), C. M. FAUQUET (7)
(1) ILTAB/ Donald Danforth Plant Science Center, St. Louis, MO, U.S.A.; (2) University of Utah, Salt Lake City, UT, U.S.A.; (3) USDA-ARS, MPPL, Beltsville, MD, U.S.A.; (4) Washington University, St. Louis, MO, U.S.A.; (5) Cornell University, Ithaca, NY, U.S.A.; (6) Oklahoma State University, Stillwater, OK, U.S.A.; (7) Donald Danforth Plant Science Center, St. Louis, MO, U.S.A.
Phytopathology 100:S154

The number of plant viruses known is increasing exponentially, and new methods such as deep sequencing will increase further their number. However, with the drastic decline in the number of expert plant virologists, virus identification becomes more challenging. DNA microarray methods based on oligonucleotide probes offer cheap, rapid, reliable, and parallel detection of plant viruses. Our preliminary data with a prototype broad range plant virus chip containing 70 mer oligonuleotide probes specific to viruses at the species or genera levels suggests the possibility to build a universal plant virus microarray (UPVM) with probes for every taxon of the plant virus kingdom. The design of 60 mer oligonucleotide probes based on taxonomic principles greatly helps not only detection/identification but also classification of known or new viruses. Here we report the first design of taxonomy-based universal plant virus microarray probes for every taxon/node of the taxonomic tree for all plant viruses available in GeneBank. A robust computational strategy will be used for objectively interpreting the resulting hybridization pattern data in an automated fashion. Preliminary *in silico* evaluation of the designed oligos will be reported to demonstrate the feasibility of using them on a chip.

High throughput sequencing - next wave diagnostics
N. BOONHAM (1), I. Adams (1), R. Glover (1), W. Monger (1), T. Hodges (2), P. Ashton (2)
(1) Food and Environment Research Agency, York, UNITED KINGDOM; (2) University of York, York, UNITED KINGDOM
Phytopathology 100:S154

Taken as a 30-year average, one new plant virus is found each year in the UK alone; these are either known viruses new to the region, variants within a known genus or new, previously un-described viruses. Detecting these new viruses is notoriously difficult, usually involving traditional investigational techniques and molecular methods such as PCR using degenerate family/genus primers. Whilst micro array techniques promised much in the area of viral diagnostics they have not yet become established as a routine tool. Microarrays have proven most useful for screening for viruses where the sequences are known and the diversity understood. Adoption is still likely if a high throughput platform can be exploited to deliver cost savings over running multiple parallel PCR tests. Development of virus discovery arrays based on probes designed at a higher taxonomic level has been disappointingly slow and results frequently need significant follow up. Next generation sequencing however offers enormous potential to simplify this work. Completely *de-novo*, generic work flows can be developed, and the volume of sequence generated (GS-FLX = 1 billion nt per run) means even low titre infections can be seen amongst the sea of host (and other pathogen) sequence. Short term the challenge is developing effective bioinformatics pipelines to 'sift' the data; longer term it is ascribing the causal agent of disease to one of the multitude of candidates discovered.

Viral population analysis by genomic sequencing
Z. XIONG (1), Z. Weng (1), D. W. Galbraith (1), W. O. Dawson (2)
(1) University of Arizona, Tucson, AZ, U.S.A.; (2) University of Florida, Lake Alfred, FL, U.S.A.
Phytopathology 100:S154

Advances in sequencing technologies have enabled unprecedented analyses of virus populations at the genomic level, to not only reveal the structure, dynamics, and sequence polymorphisms of a viral population, but also map recombination events across the entire genome. This approach is particularly suitable for viruses that infect perennial hosts and that often coexist as a complex of multiple, divergent strains, such as *Citrus tristeza virus* (CTV). We developed and applied two high-throughput techniques to the genomic analysis of CTV: an inexpensive high-density resequencing microarray, and the high coverage approach of 454 pyrosequencing. The Affymetrix resequencing microarray comprised nearly one million probes capable of interrogating genomes of known CTV strains. Analyses of natural CTV isolates indicated that the microarray readily determined major CTV strains and their prevalence within a sample, but it was ultimately limited in sequencing accuracy across the entire genome, due to cross hybridization in regions highly conserved between strains. In contrast, 454 sequencing generated high quality, full-length sequences and high resolution maps of polymorphic sites for each strain within a CTV complex. Furthermore, it identified recombinant sequences formed between constituent strains and the positions of recombination hotspots across a genome. These data together provide significant insights into the evolution and emergence of new viral strains.

Next generation sequencing as a tool for studying virus ecology
U. MELCHER (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S154

Recent and ongoing developments in nucleotide sequencing enable asking questions about the roles viruses play in ecosystems. Second generation sequencing methods enable deep sequence coverage of single genomes and metagenomes or wide sequence coverage of multiple specimens. Third generation sequencing, already emerging, offers even more extensive possibilities. A selection of newer sequencing strategies will be briefly described and their potentials assessed. The general application of such sequence approaches in ecology is to test for the presence of viruses in ecological samples and to identify those present. Detection and identification is performed bioinformatically and depends absolutely on some similarity with sequences in the nucleotide or protein databases. Strategies for detecting sequences from completely novel viruses still need to be tested. Identifying what viruses are where and when they are there is important knowledge for assessing may questions about the roles of viruses in ecology. These will be considered at three levels: within the plant, in plant and vector communities, and around the globe.

Bioinformatic analysis of microarray and next generation sequencing data
K. F. FISCHER (1)
(1) University of Utah School of Medicine, Salt Lake City, UT, U.S.A.
Phytopathology 100:S154

Unbiased direct interrogation of the nucleic acid sequences in complex biological specimens can reveal the presence of novel or unexpected agents (e.g. viruses, archaea, bacteria, or other microbes). The complexity of metagenomic and metatranscriptomic data requires efficient analysis techniques that utilize what is already known about biological sequence diversity. However, current and foreseeable sequence databases contain

enormous redundancy that can bog down analyses. Furthermore, non-random distribution of organisms found in public databases presents significant challenges. Designing and interpreting experiments that use these rich resources requires an understanding of the biases present in the existing databases and how they can affect our results. Metagenomic pathogen detection microarrays can be designed and tuned to use microarray capacity efficiently with specificity tailored to discovery, diagnostics or a compromise between those roles. Experimental bias is necessarily introduced in selecting the probes for an array. This bias can be avoided by using deep sequencing, but very similar effects are seen in the post-sequencing analysis of metagenomic DNA and RNA studies. Understanding these bioinformatics challenges has allowed broad experimental platforms to address some of the shortcomings of traditional high-specificity diagnostics.

Epidemiology/Ecology/Environmental Biology

Assuring the Safety of Fresh Produce: Issues and Strategies

Genes, genomes, and microbes; food safety research as a plant pathologist
J. BARAK (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S155

Bacterial pathogens, both plant and human, colonize crop plants and can share environmental niches. The colonization of plants by enteric animal pathogens causes a unique human public health concern. In addition to the direct cost of human illness, impacts of fresh produce-linked epidemics of food borne illness have reached the tens to hundreds of millions of dollars for each industry implicated. These outbreaks have eroded the public's faith in the healthiness of fresh produce. Plant pathologists bring a collective knowledge and experience to unravel the synergy between the plant and human pathogens on plants. Discoveries have been made in the identification of mechanisms used by the bacteria to attach to and colonize plants, as well as factors that impact the nature of such interactions. In contrast to other scientific disciplines that study food safety, the perspective of the plant pathologist centers on plants, pathogens, and the environment. The training and experience routinely applied by plant pathologists to understand how microbes colonize plants, are dispersed in the environment, and how plants defend themselves will be critical elements of a balanced program to minimize food borne illnesses.

Assessing vegetable producers' beliefs regarding food safety issues
M. LEWIS IVEY (1), J. T. LeJeune (2), S. A. Miller (2)
(1) Ohio State University, Wooster, OH, U.S.A.; (2) Ohio State University, OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S155

Foodborne disease outbreaks caused by contaminated fresh produce continue to be a concern despite widespread efforts to reduce their incidence. Knowledge gaps, misconceptions and emerging perceptions among growers regarding their decision-making processes and practices to prevent and respond to pre- and post-harvest contamination were identified using responses to a survey mailed to Midwestern vegetable growers (n=621). Returned surveys (n=261) were coded and responses analyzed using non-parametric statistical tests. Only growers who self-reported being very familiar with good agricultural practices (GAPs) implemented them consistently. There was no significant correlation between frequency of GAPs implementation, such as water and equipment sanitation, and GAPs familiarity amongst growers who claimed any lesser degree of familiarity. Growers agreed that pre-harvest plant diseases and pre- and post-harvest insects were sources of microbial contamination but were unsure if transplants and post-harvest plant diseases were contamination sources. Most growers disagreed that seeds were a source of contamination. Eminently, there is a gap in perceived knowledge between familiarity and implementation of GAPs. Growers' beliefs that plant diseases can be sources of contamination warrant further studies in plant-human pathogen interactions on produce. These findings support the development of target-specific methods of communication and response.

Seed industry challenges
R. L. DUNKLE (1)
(1) American Seed Trade Association, Alexandria, VA, U.S.A.
Phytopathology 100:S155

Food-borne illness outbreaks linked to fresh produce have led to extensive investigations by federal and state agencies to identify exact source(s) of human pathogens. To this date, no studies or investigations have linked seed planted for fresh produce production to any outbreaks in the U.S. In the wake of the outbreak of E. coli 0157:H7 in California in 2006, many dealers and growers felt compelled to test spinach, lettuce, and other types of vegetable seed for human pathogens prior to sale or planting in the U.S. Since testing began in late 2006, ASTA has not been made aware of any seed that has tested positive for human pathogens. Seed used for the production of sprouts continues to pose a risk primarily because of the potential for post harvest seed contamination during the sprout production process and the environmental conditions which favor survival and incubation of microorganisms that may have been introduced. In 2007 ASTA released and in 2010 updated a statement on testing seed for human pathogens, concluding that there is no significant value in testing seeds for the presence of human pathogens. ASTA continues to closely monitor research literature as well as all outbreaks to ensure that seed remains a negligible risk. Most seed companies have intensified their quality management programs to provide further assurrances. This presentation will discuss efforts being implemented by the seed industry to prevent seed from becoming a pathway for human pathogens.

Ground Zero: Food safety research and extension in California's Salinas Valley
S. T. KOIKE (1)
(1) University of California Cooperative Extension, Salinas, CA, U.S.A.
Phytopathology 100:S155

Coastal California and the Salinas Valley are home to the nation's most extensive leafy greens vegetable industry, where huge quantities of high quality, fresh salad commodities are grown year round. While food safety concerns are not new to this industry, the 2006 *E. coli* O157:H7 outbreak on coastal spinach significantly altered this leafy greens world. This historic event brought to light critical and unsettled issues. Foundational epidemiological gaps, such as the sources of *E. coli* and its ability to survive in the field, remain unfilled. Unproven assumptions, such as the belief that wild animals are an important source of *E. coli* O157:H7, are used to establish field practices and devise regulations. Proposed policies are based on research generated in laboratories, growth chambers, and greenhouses. To fill gaps in our knowledge of pre-harvest foodborne pathogen dynamics, county-based extension researchers in the Salinas Valley teamed with campus-based personnel and the leafy greens industry to conduct *E. coli* field studies placed in Salinas Valley fields. Soil, lettuce, and spinach were inoculated with generic and attenuated O157:H7 *E. coli* strains, plots were handled according to commercial practices, and the survival of these organisms was studied. This field-based approach demonstrated a role for off-campus extension in addressing the needs of the farming clientele and highlighted the appropriateness of field studies, conducted in real-world environments, in contributing to food safety solutions.

Collaboration, cooperation, and engagement across agencies
L. L. SKELTON (1)
(1) FDA, CFSAN, Office of Food Safety, Produce Safety Staff, College Park, MD, U.S.A.
Phytopathology 100:S155

Staff throughout USDA and FDA have been collaborating for years on various projects. However, it has recently been brought to the attention of policy-makers and administration officials that this collaboration is not well known. The presentation will focus on highlighting some of the history of past collaborative efforts, current activities and a new emphasis on engaging of other Federal and State agencies throughout government specifically related to the work FDA is undertaking on developing a produce safety rule.

The APS produce safety interagency initiative
J. FLETCHER (1)
(1) Oklahoma State University, Stillwater, OK, U.S.A.
Phytopathology 100:S155

Plant pathologists, who investigate the complex relationships between microbes and plants, have much to contribute to the discovery and design of effective solutions to microbial contamination of food plants. How microbes colonize plants and are dispersed in the environment, and how plants defend themselves, are crucial elements for developing intervention strategies and a balanced program to minimize foodborne illnesses. The effectiveness of specific risk reduction and prevention strategies is unclear since we have insufficient knowledge about the interactions of food borne pathogens with

one another, with plants, and with nonpathogenic microflora. Effective solutions will require the application of emerging research tools and strategies, as well as creative cross-disciplinary research efforts. The APS Public Policy Board proposes a focus on research to gain fundamental and practical knowledge of human pathogen-plant interactions. This should include: (1) adding fundamental and applied research to the priorities of the White House Food Safety Working Group; (2) an interdisciplinary workshop to bring all relevant members of the food safety community (agency leaders; academic, government and industry researchers; funders and regulators) together to prioritize research needs and actions; and (3) establishment of a new interagency funding initiative for fundamental and applied research on the association of human pathogens with plants.

Plant Disease Epidemics and Food Security in Globally Changing Agricultures and Environments

Food security and plant disease epidemics: Modeling potential epidemics on rice, potato, and wheat
S. SAVARY (1), E. Duveiller (2), G. Forbes (3), L. Willocquet (1), R. Hijmans (4)
(1) IRRI, Manila, PHILIPPINES; (2) CIMMYT, Mexico, MEXICO; (3) International Potato Center (CIP), Lima, PERU; (4) UC Davis, Davis, CA, U.S.A.
Phytopathology 100:S156

Climate change and increased climate variability will affect the vulnerability of major world food crops to disease epidemics. We present on-going research to link epidemiological models with crop deployment models in a spatial framework to quantify the risks that such changes are posing. We attempt to produce a coherent set of models that are robust, transparent (simple), yet which can incorporate the effects of host plant resistance characteristics, chemical protection, and other aspects of crop management. A major difficulty in this work is the paucity of actual disease observation data that could be used to validate models across large areas. Predicting future crop management is perhaps the intellectually most difficult challenge. In this presentation, we report recent progress with Potato, Wheat, and Rice, and indicate some key directions for future research.

Estimates of global crop losses
E. OERKE (1)
(1) University of Bonn, INRES - Phytomedicine, Bonn, GERMANY
Phytopathology 100:S156

Productivity of crops is at risk due to the incidence of pests, especially weeds, pathogens and animal pests. Crop losses to pests can be substantial and may be reduced by various control activities. Estimates on the loss potential and actual losses - despite of current crop protection practices – are given for major food and cash crops on a regional level as well as for the world-wide total. Among crops the total loss potential of pests world-wide varies from about 50% to >80%; actual losses vary from 25 to 40%. Overall weeds have a higher loss potential than animal pests and pathogens. Efficient control of pathogens and animal pests is more complex than weed control which can be managed mechanically or chemically, and largely relies on the use of synthetic chemicals. The efficacy of crop protection is higher in cash crops than in food crops; differences among regions are more pronounced for food crops. Despite an increase in pesticide use crop losses have not been significantly decreased during the last decades. Pesticide use has enabled farmers to modify production systems and to increase crop productivity without sustaining higher losses likely to occur from an increased susceptibility to pests. Minor losses often are economical acceptable, however, an increase in crop productivity without adequate disease control is not cost-effective, because a raise of the site-specific yield potential is often associated with an increased vulnerability to damages inflicted by pathogens.

Seeking impact on food security of the poor through phytopathological science
R. J. NELSON (1)
(1) Cornell University, Ithaca, NY, U.S.A.
Phytopathology 100:S156

Plant diseases reduce crop productivity and quality on a chronic or intermittent basis, and have sometimes contributed to famines. With changes in climate, threats to food security may be exacerbated. This presentation will take a comparative view of the challenges and opportunities for disease management in relation to the food security of resource-poor farmers, based on the author's experience with pathosystems involving staple crops in Asia, Latin America and Africa. The features of several pathosystems (rice blast, potato late blight and northern corn leaf blight and mycotoxin-producing ear rots) will be considered in relation to the use of host resistance and other measures. The problematic links between genetic analysis, resistance breeding and deployment of resistance will be considered for the three diseases. Management of *Aspergillus* ear rot in maize, which is associated with a potent mycotoxin (aflatoxin), is particularly difficult since it can have substantial public health implications without presenting obvious symptoms. Lessons learned across the pathosystems, both shared and disease-specific, will be highlighted.

The role of pest risk analysis and quarantine measures in food security
P. H. BERGER (1), C. Devorshak (2)
(1) Center for Plant Health Science & Technology, Raleigh, NC, U.S.A.; (2) Center for Plant Health Science & Technology, Plant Epidemiology & Risk Analysis Laboratory, Raleigh, NC, U.S.A.
Phytopathology 100:S156

Food security for much of the world remains a precarious situation. Threats to food security include economic, political and biological factors. Plant diseases have the potential to impact food security, by reducing food supply, and also through affecting livelihoods and economic stability. Diseases may spread from one place to another naturally, or through human mediated spread. Global trade in agricultural products and the movement of staples food aid present some of the biggest challenges in the spread of plant diseases to new places. One of our most important defenses in protecting our food supply is our ability to identify and analyze threats—both existing and emerging, to determine pathways of spread, model infection, predict impacts and formulate recommendations for appropriate actions, in advance of a potential introduction or epidemic. We are constantly refining analytical techniques used to analyze risks associated with both existing and emerging plant diseases, including economic and biological modeling, climate based mapping, and surveillance. At the same time, improved access to scientific information, in some cases through "real time" feeds, further aids our ability to analyze potential threats, and in some cases prevent serious impacts from occurring, or allowing us to take more effective action to lessen the magnitude of impacts.

Plant Pathogen Population Genetics: An Essential Tool for Crop Biosecurity

How can population genetics inform crop biosecurity efforts?
N. J. GRUNWALD (1)
(1) USDA ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S156

In an ever more interconnected world, bioinvasions of new species, clones or populations of plant pathogens are increasing in importance. Novel migrant plant pathogens have repeatedly emerged as threats to agricultural, forest and other ecosystems. Population genetic theory and tools have an important role to play in characterizing the pattern and process of bioinvasions. Appropriate molecular marker systems need to be combined with suitable methods for analyzing population structure given the data. Tools for asking specific evolutionary questions, particularly using coalescent theory, are expanding rapidly. Population genetic approaches can for example assess if immigrant populations are sexually recombining, subject to a bottleneck, or migrating from one or several source populations.

Application of comparative genomics for the identification and monitoring of the highly virulent African race, Ug99, of *Puccinia graminis*
J. CROUCH (1), S. Sakthikumar (2), C. Cuomo (2), S. Stoxen (3), Z. A. Pretorious (4), L. J. Szabo (1)
(1) Cereal Disease Laboratory, USDA-ARS, St. Paul, MN, U.S.A.; (2) Broad Institute, Cambridge, MA, U.S.A.; (3) University of Minnesota, St. Paul, MN, U.S.A.; (4) University of the Free State, Bloemfontein, SOUTHWEST AFRICA
Phytopathology 100:S156

Throughout human history, wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) has been one of the most destructive diseases of cereal crops. Stem rust has been well controlled in the U.S. for nearly a half a century, but with the appearance of a new, highly virulent race of Pgt in Uganda ("Ug99"), this disease has reemerged as a serious threat to global food supplies. Ug99 has already spread throughout northeastern Africa, the Arabian Peninsula and recently into Central Asia, and is predicted to move into North America within 10 years, likely through human mediated activities. To date five members of the Ug99 lineage have been found in Africa, showing variation in

virulence to the stem rust resistance genes *Sr24*, *Sr31* and *Sr36*. Over 1 million SNPs were identified by mapping Illumina sequence data from five members of the Ug99 lineage as well as three additional isolates against the assembled Pgt reference genome. This SNP database was used to selectively design 38 real-time PCR hydrolysis probes, each containing at least two homozygous SNPs. By screening a worldwide collection of 270 Pgt isolates, we found that individually the probes were not capable of uniquely discriminating between Ug99 and other races. Nevertheless, when a suite of probes was used in combination, a distinct Ug99 fingerprint pattern was generated. Ultimately, this technology will be a key component in monitoring the movement of Ug99.

Inference of *Phytophthora ramorum* migration pathways
E. M. GOSS (1)
(1) USDA ARS, Corvallis, OR, U.S.A.
Phytopathology 100:S157

Population genetic analysis can reveal important insights into the movement of plant pathogens, such as the connectivity of populations, migration events among geographic regions, and the tracing of new epidemics to an inoculum source. *Phytophthora ramorum*, the sudden oak death pathogen, was first detected in California and Europe in the early 1990's. The pathogen has caused extensive mortality to coast live oak and tanoak in coastal Northern California and has been costly to ornamental nurseries found to have *P. ramorum*-infected plants. Human activities have repeatedly contributed to the spread of this pathogen and long-distance migration of the pathogen is linked to the plant trade. Population genetic analysis has been integral to tracking its movement to date. Population genetic-based inferences on domestic and global *P. ramorum* migration events will be discussed.

The population genomics of mycotoxin diversity in *Aspergillus flavus* and *Aspergillus parasiticus*
I. CARBONE (1), B. W. Horn (2), G. G. Moore (1), R. A. Olarte (1), C. J. Worthington (1), J. T. Monacell (3), E. A. Stone (4)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.; (2) National Peanut Research Laboratory, USDA, ARS, Dawson, GA, U.S.A.; (3) Department of Plant Pathology and Bioinformatics Research Center, North Carolina State University, Raleigh, NC, U.S.A.; (4) Department of Genetics and Bioinformatics Research Center, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S157

Mycotoxins, and especially the aflatoxins, are an enormous problem in agriculture, with aflatoxin B1 being the most carcinogenic known natural compound. The worldwide costs associated with aflatoxin monitoring and crop losses are in the hundreds of millions of dollars. *Aspergillus flavus* and *A. parasiticus* are the most common agents of aflatoxin contamination of oil-rich seed and grain crops. In addition to aflatoxins, *A. flavus* produces another unrelated mycotoxin, cyclopiazonic acid (CPA). Populations of *A. flavus* show a high level of variation in mycotoxin production, with individuals producing both aflatoxins and CPA, aflatoxins alone, CPA alone or neither mycotoxin. We performed array-Comparative Genome Hybridization (aCGH) for multiple sexual crosses in *A. flavus* and *A. parasiticus* whereby each cross was represented as a parent-offspring trio comprising two parents and one offspring. Examination of aCGH data for each parent-offspring trio revealed that meiotic recombination is highly structured along chromosomes, with recombination hotspots in the aflatoxin, CPA and other predicted secondary metabolic gene clusters. Crossovers in the aflatoxin cluster of F1 progeny coincided with recombination hotspots observed in natural populations. Population genetic data show that a combination of ecological factors, asexual/sexual reproduction and balancing selection may influence mycotoxin diversity in these agriculturally important fungi.

A population genetics framework for understanding aggressiveness and toxigenicity of Fusarium head blight pathogens
T. WARD (1)
(1) USDA ARS, Peoria, IL, U.S.A.
Phytopathology 100:S157

A 14-fold increase in the frequency of 3ADON-producing F. graminearum occurred between 1998 and 2004 in western Canada. Significant population structure associated with trichothecene chemotype differences was observed, and isolates from the 3ADON populations were found to accumulate significantly more trichothecene than 15ADON populations. 3ADON populations also exhibited higher fecundity and growth rates. Expanded molecular surveillance based on 4,266 F. graminearum isolated from seven Canadian provinces between 2005 and 2007 demonstrated the trichothecene chemotype distribution in Canada was characterized by two longitudinal clines. The frequency of 3ADON isolates continued to increase significantly in western Canada between 2005 and 2007. However, similar changes in chemotype frequency among isolates from eastern Canada were not observed. These data suggest a difference in selective pressure acting on FHB pathogens in eastern and western Canada or an uncoupling of the 3ADON chemotype from the trait or traits under selection in some eastern Canadian FHB populations. Analyses of the global population structure of F. graminearum revealed a very close genetic relationship between a Japanese 3ADON population and the novel 3ADON populations in North America. Additional evidence of transcontinental movement of populations followed by limited genetic exchange between resident and introduced populations is presented.

Molecular/Cellular/Plant-Microbe Interactions

Broad-Spectrum Resistance: Molecular Mechanisms Involved in Pathogen Reception and Resistance Signaling

Pattern recognition receptors in plant innate immunity
C. J. RIDOUT (1)
(1) John Innes Centre, Norwich, UNITED KINGDOM
Phytopathology 100:S157

Plants have pattern recognition receptors (PRRs) that detect pathogen associated molecular patterns (PAMPs) such as fungal chitin and the bacterial protein EF-Tu. Since PAMPs are highly conserved across microbial species, taxa and kingdoms, PRRs could potentially offer durable broad-spectrum resistance. We are investigating how PAMP-triggered Immunity (PTI) contributes to partial resistance in agricultural crops. Working with wheat, barley and oilseed rape (canola), we are evaluating natural variation in PTI responses including the induction of early reactive oxygen species (ROS), defence genes and callose formation. Wide variation in ROS production has been established in Brassica parental mapping lines responding to chitin. Pathology tests will now be performed to establish whether there is a relationship between PTI and quantitative disease resistance loci. ROS burst also varies in barley accessions, but has not been detected in wheat. EF-Tu receptor (EFR) is a PRR first identified in Arabidopsis (Zipfel et al, 2006, Cell 125: 764). ELF18, an 18-amino acid peptide from EF-Tu, is sufficient to elicit PTI responses. EFR was successfully transferred to tomato, which gained the response to ELF18 (Lacombe et al, 2010, Nature Biotech in press). We have now transformed EFR into wheat, and testing whether there is a gain in ELF18 response. Our research will enable us to evaluate the potential of PRRs for developing broad-spectrum disease resistance in agriculture.

Dissecting QTL: The genes that contribute to disease resistance revealed
J. E. LEACH (1), R. M. Davidson (1), J. Snelling (1), M. Bruce (1), H. Leung (2), C. M. Vera Cruz (1)
(1) Colorado State University, Fort Collins, CO, U.S.A.; (2) International Rice Research Institute, Manila, PHILIPPINES
Phytopathology 100:S157

Incorporation of quantitative trait loci (QTL) that control disease resistance into elite germplasm could help reduce crop losses, and may even confer broad-spectrum and durable resistance. Applied breeding programs, however, have not enthusiastically pursued QTL for crop improvement. A key reason for this lack of adoption is that reliable markers for accumulation of the QTL are not readily available because the genes that contribute to the quantitative trait are not known. Identifying these genes that function in QTL phenotypes has proven difficult partly because of the imprecision of QTL mapping and because the effects are small and can vary with environment. In rice, the availability of a high quality genome sequence and the ability to associate several types of phenotypic data, including transcript information, to the physical map, is allowing new approaches to link complex phenotypes to genomic regions and even genes. Progress in the identification of genes contributing to rice disease resistance QTL, and description of future approaches to expedite gene and marker identification for use in breeding programs will be discussed.

Genetical genomics of Ug99 stem rust infection identifies master regulators of defense in barley
M. Moscou (1), N. Lauter (2), B. Steffenson (3), R. WISE (2)
(1) Iowa State University, Ames, IA, U.S.A.; (2) USDA-ARS / Iowa State University, Ames, IA, U.S.A.; (3) University of Minnesota, St. Paul, MN, U.S.A.
Phytopathology 100:S157

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a devastating fungal disease with a host spectrum that includes wheat, barley, and its alternate host, common barberry. Resistance in barley to *P. graminis* f. sp. *tritici* race TTKSK (*Pgt* Ug99), a race with novel virulence, is mediated by the *Rpg-TTKSK* locus on chromosome 5H that contains the known resistance genes *rpg4* and *Rpg5*. Variation in resistance observed on young plants of the Q21861 x SM89010 (QSM) doubled haploid (DH) population is predominantly a qualitative phenotype, with little of the remaining variance explained by loci other than *Rpg-TTKSK*. In contrast, infection phenotypes assayed on adult QSM DH plants infected by field inoculum of *Pgt* Ug99 in Njoro, Kenya found several additional quantitative trait loci that contribute to resistance. To molecularly characterize these loci, Barley1 GeneChips were used to measure expression of 22,792 genes in the QSM population after treatment with *Pgt* Ug99 or with mock-inoculation. By analyzing the changes in genomic distributions of expression Quantitative Trait Loci (eQTL) between treatments, we identify a chromosome 2H *trans*-eQTL hotspot that regulates the expression of hundreds of inoculation responsive genes scattered around the genome. This 2H.17 locus coincides with an enhancer of adult plant *R*-gene-mediated resistance discovered through the Njoro field trials and provides early transcriptional targets of *Rpg-TTKSK*-mediated resistance to *Pgt* Ug99.

Targeting conserved effectors and effector motifs for broad spectrum resistance against oomycetes and fungi

B. M. TYLER (1), S. D. Kale (1), B. Gu (2), D. G. Capelluto (3), D. Dou (4), F. Arredondo (1), D. Deb (5), R. Anderson (5), V. Antigliani (1), I. Fudal (6), T. Rouxel (6), J. McDowell (5), C. Lawrence (1), W. Shan (2)
(1) Virginia Tech, Virginia Bioinformatics Institute, Blacksburg, VA, U.S.A.; (2) Northwest A & F University, Yangling, PRC PEOPLES REP OF CHINA; (3) Virginia Tech, Dept. Biological Sciences, Blacksburg, VA, U.S.A.; (4) Department of Plant Pathology, Nanjing Agricultural University, Nanjing, PRC PEOPLES REP OF CHINA; (5) Virginia Tech, Dept. Plant Pathology, Physiology and Weed Science, Blacksburg, VA, U.S.A.; (6) INRA-Bioger, Versailles, FRANCE
Phytopathology 100:S158

Fungal and oomycete pathogens of both plants and animals produce effectors and/or toxins that act within the cytoplasm of host cells to suppress host defenses and cause disease. Effector proteins of oomycete plant pathogens utilize an N-terminal motif, RXLR, to enter host cells. Genome sequences of oomycete pathogens have revealed hundreds of RXLR effectors. Most of these are rapidly evolving, but a small number are highly conserved, suggesting they may be good targets for broad-spectrum R genes. By detailed mutagenesis of oomycete RXLR motifs, we defined a new bioinformatic model for these motifs and used it to identify candidate RXLR-like motifs in fungal effectors. Several of these motifs were then confirmed experimentally to be responsible for cell entry by these effectors. We have found that both oomycete and fungal RXLR(-like) motifs are responsible for binding of the effectors to phosphatidyl-inositol-3-phosphate (PI-3-P). Using this information, we have succeeded in blocking entry of oomycete and fungal effectors into host cells by using the head-group mimic inositol-1,3-diphosphate or by using PI-3-P-binding proteins. These findings suggests that agrochemicals that mimic inositol-1,3-diphosphate, or transgenes that encode secreted PI-3-P binding proteins, may confer broad-spectrum protection against oomycete and fungal infection.

The molecular basis of broad-spectrum powdery mildew resistance

R. PANSTRUGA (1), C. Consonni (1), M. Humphry (1), J. Lorek (1), K. Becker (1), P. Bednarek (1)
(1) Max-Planck Institute for Plant Breeding Research, Köln, GERMANY
Phytopathology 100:S158

Loss-of-function mutant alleles of the barley *Mlo* locus are known to confer durable, broad spectrum resistance against the powdery mildew disease caused by the Ascomycete *Blumeria graminis* f. sp. *hordei*. This type of antifungal immunity has been discovered 65 years ago and has been widely used in European agriculture for more than 25 years. We recently showed that powdery mildew resistance conferred by *mlo* alleles is not restricted to barley, but also occurs in *Arabidopsis*, tomato and pea. The molecular basis of this unusual type of disease resistance remains, however, mysterious. We exploit the genetic and molecular tools available for the dicot reference species, *Arabidopsis thaliana*, to get insights into the molecular mechanisms leading to *mlo* resistance. We propose a model in which powdery mildew fungi exploit MLO protein function for fungal pathogenesis by suppressing multiple defence pathways in their plant hosts.

More than Just Antibiotics: The Multiple Mechanisms Leading to Biological Control and Plant Growth Promotion

The multiple roles of auxin production and turnover in bacteria: Impact on plant health

J. LEVEAU (1)
(1) University of California - Davis, Davis, CA, U.S.A.
Phytopathology 100:S158

Many plant-associated bacteria have evolved ways to tap into plant hormone signalling pathways and to manipulate plant physiology accordingly and to their own advantage. One of the mechanisms for this exploitation of the plant hormone system is the bacterial synthesis or destruction of one or more of the five classical plant hormones, i.e. auxin (indole 3-acetic acid or IAA), ethylene, abscisic acid, cytokinin and gibberellin. Our understanding of the pathways, genes, and enzymes underlying bacterial tampering with plant hormone homeostasis is biased towards what is known about a small number of intensively studied cases, e.g. the synthesis of IAA, which may be beneficial or detrimental to plants, or the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which lowers inhibitory ethylene concentrations through degradation of the ethylene precursor ACC. Less is known about other bacterial activities, such as the phenomenon of IAA turnover which has long been recognized to exist but remains unexplained as to its effects, if any, on plant physiology. Only recently, the first bacterial genes for IAA turnover were discovered and characterized in a Pseudomonas species. This presentation will cover the most recent findings in the field of bacterial IAA turnover and explore the various ways in which this bacterial phenotype might be exploitable towards the biological control of plant pathogens and/or the promotion of plant growth and health.

Bacterial determinants of induced systemic resistance induced by *Pseudomonas chlororaphis* O6

S. HAN (1), A. J. Anderson (2), B. McSpadden Gardener (3), K. Yang (1), Y. Kim (1)
(1) Chonnam National University, Gwangju, KOREA; (2) Utah State University, Logan, UT, U.S.A.; (3) Ohio State University, Wooster, OH, U.S.A.
Phytopathology 100:S158

Root colonization by the beneficial rhizobacterium, *Pseudomonas chlororaphis* O6, induces disease resistance in tobacco against two different leaf pathogens. To identify the bacterial determinants involved in resistance, we used mutational and biochemical analysis. By screening transposon-generated mutants in *P. chlororaphis* O6 for a reduced potentional to induce resistance in tobacco to the soft-rot pathogen, a diverse set of genes involved was implicated in resistance, including those involved in chemotaxis, biosynthesis of purine, phospholipase C, transport of branched-chain amino acids, an ABC transporter, and the two-component sensor kinase GacS. Additional mutations were detected in the intergenic spacer regions between genes encoding a GGDEF protein and fumarate dehydratase, and in genes of unknown function. Biochemical studies indicated that a *P. cholroraphis* O6 produced 2R,3R-butanediol as an active compound to induced systemic resistance against tobacco soft rot disease but not wildfire disease. Treatment of tobacco with the pure compound also enhanced aerial growth and induced tolerance to abiotic stresses, a phenomenon also seen in the plants that were colonized by *P. chlororaphis* O6. The global sensor kinase, GacS, of *P. chlororaphis* O6 was a key regulator for induced systemic resistance against *E. carotovora* through regulation of 2R,3R-butanediol production. This is the first report of microbial genes related the induced systemic resistance and a volatile production by pseudomonad.

Differential roles of lipopeptides in plant host defenses and pathogen suppression

M. ONGENA (1), G. Henry (1), P. Thonart (1)
(1) University of Liege, Gembloux Agro-Bio Tech, Gembloux, BELGIUM
Phytopathology 100:S158

Cyclic lipopeptides (cLPs) constitute a structurally diverse group of metabolites produced by various fungal and bacterial genera. Recent advances revealed that the panel of natural functions retained by these lipopeptides is larger than previously suspected. Focusing on cLPs isolated from *Pseudomonas* and *Bacillus*, we will provide an overview of this structure-related versatility of their biological functions. cLPs from plant-associated bacteria may be virulence factors synthesized by pathogenic isolates but may also play a crucial role in the biocontrol activity of beneficial strains because of their involvement in motility, biofilm formation and pathogen antagonism. In addition, some rhizobacterial cLPs such as surfactin and massetolide tightly interact with plant cells and stimulate the induced systemic resistance (ISR) at the micromolar level. They constitute a novel class of bacterial elicitors with a

possibly specific mechanism of action that we wanted to further investigate. Rather than a receptor-mediated recognition process, our results suggests that surfactins prefererably interact with the lipid fraction of the plant plasma membrane. These cLPs do not create irreversible pore formation but act in a way sufficient to induce some disturbance or transient channelling in the phospholipid bilayer that can in turn activate a biochemical cascade of molecular events leading to defensive responses.

Bacterial signaling: Activation of secondary metabolites with multiple roles in biological control

L. S. PIERSON (1), E. A. Pierson (2)
(1) Texas A&M University, College Station, TX, U.S.A.; (2) University of Arizona, Tucson, AZ, U.S.A.
Phytopathology 100:S159

Most bacteria produce and detect small signal molecules. These signals allow bacteria to coordinate specific behaviors such as swimming, swarming, exopolysaccharide production, biofilm formation, and products that contribute to either pathogenesis or symbiosis. In Gram negative bacteria, the most common class of signal molecules are the N-acyl homoserine lactones (AHLs) involved in a cell density-dependent form of gene regulation termed quorum sensing (QS). Most QS systems are composed of a gene that encodes the AHL synthase and a gene that encodes the protein that recognizes the AHL and responds by activating or repressing target gene expression. These QS systems are often integral members of complex regulatory networks themselves subject to additional levels of regulation, including transcription rates, mRNA stability, the rate of AHL synthesis and the signal threshold required for perception. The root-associated bacterium Pseudomonas chlororaphis strain 30-84 produces two major phenazine (PZ) compounds, phenazine-1-carboxylic acid (PCA) and 2-hydroxy-phenazine-1-carboxylic acid (2OHPCA). The PhzR/PhzI QS system directly regulates PZ production in strain 30-84 and is responsible for pathogen inhibition and persistence of strain 30-84 in the rhizosphere. In nature, bacteria are usually located in surface-attached communities termed biofilms. The PhzR/PhzI QS system is absolutely required for biofilm formation by strain 30-84. However, it appears PZ production under PhzR/PhzI control is a primary determinant of biofilm structure, as the presence of extra copies of phzR/phzI failed to restore biofilm formation, while constitutive expression of the PZ biosynthetic genes resulted in earlier biofilm formation. Many bacteria produce PZs, but often different derivatives and in different ratios. To investigate why this might be, we altered the overall ratios of PZs produced by strain 30-84. These changes significantly affected cell adhesion, biofilm architecture and dispersal rates. The PZ-altered derivatives also differed in their ability to inhibit plant pathogens in vitro. These effects of PZs on biofilm development are probably due to multiple mechanisms, including by changing cell surface properties and by affecting changes in patterns of gene expression. We will discuss how PZs may themselves serve as signaling molecules to alter gene expression and the effect of non-PZ producers in mixed community biofilms.

Redefining the paradigm of biocontrol

B. MCSPADDEN GARDENER (1), S. Park (1), C. Chunxue (1), X. Rong (1)
(1) The Ohio State University - OARDC, Wooster, OH, U.S.A.
Phytopathology 100:S159

Contemporary research has indicated the multifunctional nature of various microbial components in the phytosphere. At the molecular level, different microbially-associated molecular patterns are known to influence host plant physiology in rather dramatic ways. Lipopeptides and polyketides, once thought to be nothing more than antifungal metabolites, have been shown to be important factors in the induction of host resistance, plant stress resistance, and biofilm formation. At the population level, some microbial populations may affect disease development in contrasting ways, depending on the structure of plant associated microbial communities and/or the prevailing abiotic conditions they are experiencing. Given that plants are covered with diverse and ever-changing populations of microbes, host plant health status is clearly a dynamic property. Because such observations are inconsistent with the classical definitions of biological control and plant disease, a new, more holistic, perspective is required to understand the true nature of plant-microbe interactions. Such a perspective needs to take into account the diverse and dynamic nature of plant-associated microorganisms, the multifunctional nature of microbial secretions, and portray plant health as an emergent property. To that end, it is suggested that the relative importance of non-pathogenic plant-associated microbes to plant health be highlighted, a move that will lead us towards a new paradigm for biocontrol.

Nature's Molecular Biologist: *Xanthomonas* and TAL Effector Function, Structure, and Diversity

Nature's molecular biologist: *Xanthomonas* and the AvrBs3-related family of transcription activation-like (TAL) type III effectors

F. WHITE (1)
(1) Department of Plant Pathology, Kansas State University, Manhattan, KS, U.S.A.
Phytopathology 100:S159

Bacterial blight of rice represents a robust system for understanding the interaction and co-adaptation process of a bacterial pathogen and the host. *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial blight, depends on a type III secretion system for effective invasion and colonization of the host and is particularly noteworthy for the dependence on transcription activation-type (TAL) type III effector genes. Considerable progress has been made in our understanding of TAL effector function since the identification of the type effector AvrBs3. The effectors are highly conserved, although each member is distinguished by a unique configuration of the central repetitive region. The repetitive region is associated with the phenotypic activity and targeted DNA elements in the host genome. TAL effector genes are found primarily in species of *Xanthomonas*, although related genes have been identified in other species. This talk will address the basic properties of TAL effectors, their involvement in a variety of plant diseases, and their position within the pantheon of type III effectors.

Diversity of S and R genes in rice targeted by the TAL effector genes of *Xanthomonas oryzae* pv. *oryzae*

B. YANG (1)
(1) Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S159

Bacterial blight of rice is controlled in large through the molecular interaction between factors in host rice and pathogen *Xanthomonas oryzae* pv. *oryzae*. The factors in the pathogen include a large repertoire of transcription activator like (TAL) type III effectors which are, in a few cases, matched by the genes in host. One aspect of the interaction involves the recognition of TAL effectors (avirulence) by the cognate resistance (R) genes followed by the onset of strong and rapid defense responses including hypersensitive reaction (HR), so called gene-for-gene resistance, leading to an incompatible reaction of the disease. This incompatibility is exampled by the combination of *Xa27*/AvrXa27. The interaction also involves targeting of host genes by some TAL effectors (virulence) resulting in disease susceptibility provided plant lacks the gene-for-gene resistance. In either case, the host gene is transcriptionally activated by the corresponding TAL effector, and the induction is phenotypically associated with the disease susceptibility or resistance dependent on the genetic context. In cases of some susceptibility (S) genes, rice has evolved the alleles that are unresponsive to the corresponding TAL effector(s) and occur as recessive resistance genes in certain cultivars. This is illustrated by the recessive resistance gene *xa13* and its allelic *Os8N3* susceptibility gene with the corresponding TAL effector PthXo1, as well as by other S genes *Os11N3* and *Os12N3* with their respective TAL effectors.

TAL effector-driven host gene expression shapes *Xanthomonas* interactions with crop plants

A. J. BOGDANOVE (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, U.S.A.
Phytopathology 100:S159

Recent elucidation of the basis for specificity in host gene targeting by transcription activator like effectors (TALEs) of *Xanthomonas* allows the prediction of candidate targets in whole genome sequences. Combined with genome-wide transcript profiling and functional analysis, this is a powerful approach to discovering host genes and processes that can be subverted by the pathogen to promote infection. Isolation of genes for resistance in some cases can be expedited as well. Yet computational challenges to accurate predictions persist. This presentation will describe ongoing efforts to identify important rice genes in the "regulome" activated by the scores of TALEs present in two strains of the rice pathogens *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. The results of a bioinformatic study to improve binding site prediction will also be presented. Finally, implications for discovery in other major crop affected by *Xanthomonas* spp. that depend on TALEs will be discussed.

Exploiting TAL effector diversity for biotechnology

T. LAHAYE (1)
(1) Ludwig-Maximilians-University Munich, Martinsried, GERMANY
Phytopathology 100:S159

Plant pathogenic bacteria of the genus *Xanthomonas* inject Transcription-Activator Like Effector proteins (TALEs) into plant cells. Upon injection,

TALEs translocate to the plant nucleus, bind to defined DNA boxes and activate expression of the downstream host genes. TALE-mediated activation of plant promoters in most cases favours disease progression but triggers in some plant genotypes activation of the plant immune system. Recent studies uncovered the molecular basis of how the TALE DNA binding motif binds to matching DNA boxes. Using this "TALE-CODE" we have generate a designer TALE (dTALEs) that activate a desired target promoter. Recent progress on the application of dTALEs will be presented.

Small Molecules in Phytopathology: From Determinants of Disease to Modulators of Defense

Unraveling the site- and mode-of-action of protein host-selective toxins
L. M. CIUFFETTI (1), V. A. Manning (1), I. Pandelova (1), M. Figueroa Betts (1)
(1) Oregon State University, Corvallis, OR, U.S.A.
Phytopathology 100:S160

Some necrotrophic fungal pathogens produce host-selective toxins (HSTs) as major virulence/pathogenicity determinants. Diseases caused by these pathogens often follow an inverse gene-for-gene model where toxin production by the pathogen and a single, corresponding, genetically dominant locus in the host are both required for compatibility (the occurrence of disease). *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat, represents a model pathogen for studying the biological significance of the inverse gene-for-gene interaction due to its production of multiple HSTs. Ptr ToxA (ToxA) and Ptr ToxB (ToxB) are two structurally unrelated proteinaceous HSTs that evoke different host responses, yet confer pathogenicity. Comparative analyses of ToxA and ToxB reveal differences and commonalities in the mode-of-action of these effectors and thus, provide insights into the mechanism of *P. tritici–repentis*-induced disease. The presentation will center on what is known about the site- and mode-of-action of ToxA and ToxB.

Hijacking and manipulation of host responses by pathogen-derived hormone mimics: An update on functional role of coronatine in bacterial speck disease
R. UPPALAPATI (1), Y. Ishiga (1), T. Ishiga (1), T. Wangdi (1), C. Ryu (1), K. S. Mysore (1)
(1) The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.
Phytopathology 100:S160

Plants utilize phytohormones to mediate local and systemically acquired immune responses to defend against multiple pathogens. However, some pathogens have evolved mechanism of virulence to highjack the hormone-mediated signaling by producing hormone mimics to cause disease. Several pathovars of *Pseudomonas syringae* produce a chlorosis-inducing virulence factor coronatine (COR), which functions as a phytohormone mimic of methyl jasmonate (MeJA) and JA-isoleucine (JA-Ile). A comparison of COR- and MeJA-regulated transcriptomes revealed that COR and MeJA share similar, but not identical activities and impact multiple phytohormone pathways in tomato. COR, by structurally mimicking JA-Ile, hijacks an ubiquitin E3 ligase of the $SCF^{coi1/jai1}$ complex to activate JA signaling and thereby suppress salicylic acid (SA)-mediated defense responses. Furthermore, requires SGT1 (suppressor of G2 allele of Skp1) which associates with SCF complexes to induce chlorosis. Interestingly, SGT1 also regulated disease associated necrotic cell death. In an effort to elucidate the genes involved in COR-induced chlorosis and cell death, we utilized forward genetic screens and identified a role for chloroplast localized Peroxiredoxin and NADPH-dependent thioredoxin reductase in modulating COR-induced chlorosis development and disease associated necrotic cell death. Thus, COR may function at early stages of pathogenesis to suppress basal immunity and targets chloroplast to regulate disease-associated necrotic cell death.

***Sclerotinia sclerotiorum* via oxalic acid creates a reducing environment in the host which is required for pathogenic success**
M. DICKMAN (1), M. Kabbage (1), B. Williams (1), H. Kim (1)
(1) Texas A&M University, College Station, TX, U.S.A.
Phytopathology 100:S160

Necrotrophs, which require dead host tissue in order to obtain nourishment, were initially thought to cause disease symptoms and host cell death by sheer brute force via the secretion of toxic metabolites. Recently however, emerging data from several pathosystems have suggested that some necrotrophic fungi are tactically more subtle in the manner by which pathogenic success is achieved, though the mechanistic details are not known. *Sclerotinia sclerotiorum* (Ss) is an extremely broad host range (>400 species) necrotrophic fungal plant pathogen that produces the non-specific phytotoxin and pathogenicity factor, oxalic acid (OA). Transgenic plants expressing a redox-regulated GFP reporter, provided real-time evidence that Ss initially induces reducing conditions that suppresses the host oxidative burst and callose deposition, but subsequently Ss/OA promotes plant ROS generation leading to programmed cell death; of benefit of the pathogen. Our non-pathogenic OA-mutant strain is unable to alter host redox status, however, chemical induction of reducing conditions in host cells with DTT, remarkably restores its ability to cause disease. OA thus appears to have dual opposing functions, by creating reducing conditions, OA inhibits the plant oxidative burst defense response and cell death, and then subsequently promotes cell death and disease via ROS. The reduction of the host cellular environment may be a key strategy for establishment of necrotrophic fungal infection.

Azelaic acid: A new player in priming plant defense
J. T. GREENBERG (1), H. Jung (2), T. Tschaplinski (3)
(1) University of Chicago, Chicago, IL, U.S.A.; (2) Dong-A University, Busan, KOREA; (3) Oak Ridge National Lab, Oak Ridge, TN, U.S.A.
Phytopathology 100:S160

Plants produce numerous small molecules that function to regulate plant defenses. In addition to directly activating defenses, some signals act by priming defenses. In this scenario, when an infection occurs, defense responses precede more rapidly and/or more strongly. Azelaic acid is made upon infection and can translocate to distal leaves where it primes the production of the key defense regulator salicylic acid (SA). New data suggests that azelaic acid is effective both in Arabidopsis (where it was first described as having a defense priming role) and solanaceous plants. Furthermore, at least one additional small molecule is implicated as acting down stream of azelaic acid in priming SA production. Thus, plants employ numerous small molecules to initiate, activate and/or amplify defense signaling in response to infection.

Networking by small-molecule hormones in plant immunity
A. LEON-REYES (1), D. Van der Does (1), A. Koornneef (1), S. C. Van Wees (1), C. M. Pieterse (1)
(1) Utrecht University, Utrecht, NETHERLANDS
Phytopathology 100:S160

Plants live in complex environments in which they intimately interact with a broad range of pathogens and insects. Genomics approaches expanded our understanding of the molecular mechanisms by which plants tailor their immune response. Hormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play pivotal roles in the regulation of the defense signaling network (Pieterse et al., 2009: *Nature Chem. Biol.* 5: 308-316). Their signaling pathways cross-communicate, providing the plant with a powerful capacity to finely tailor its immune response to the attacker encountered. Research on the kinetics and mechanisms underlying SA-JA crosstalk revealed that the SA-JA antagonism is conserved among Arabidopsis accessions, highlighting the importance of this mechanism for plant survival. The kinetics of SA and JA signaling appears to play an important role in the outcome of the SA-JA interaction (antagonistic, synergistic, or neutral). The antagonistic effect of SA on JA-responsive gene expression is linked to SA-induced changes in the cellular redox potential, suggesting that cross-talk is redox regulated. Several key regulators involved in cross-talk have been identified, including the redox-sensitive protein NPR1. ET appears to act as an important modulator of NPR1 function in SA-JA crosstalk. Furthermore we showed that SA-mediated suppression of JA signaling acts downstream of SCF-COI1-JAZ components of the JA pathways and is directly targeted at GCC-box containing promoters of JA-responsive genes.

Small molecule inhibitors of type III secretion systems in Gram-negative plant and animal pathogens
H. B. FELISE (1), H. V. Nguyen (1), K. C. Barry (1), P. A. Bronstein (2), T. Kline (1), S. I. Miller (1)
(1) University of Washington, Seattle, WA, U.S.A.; (2) USDA, Ithaca, NY, U.S.A.
Phytopathology 100:S160

Bacterial virulence mechanisms are potential targets for drug discovery as they are required for numerous global infectious agents to cause disease. Gram-negative bacterial type III secretion systems, which are used by numerous plant and animal pathogens to deliver protein virulence effectors to host cells, may comprise one such therapeutic target. We have developed and performed high-throughput screens of small molecule libraries to identify broad-spectrum inhibitors of bacterial secretion systems by targeting conserved components of these systems. Currently, we have identified and characterized a class of compounds, 2-imino-5-arylidene thiazolidinones that

block secretion and virulence functions in a number of plant and animal pathogens, including *Pseudomonas* spp., *Yersinia* spp., and *Salmonella enterica* serovar Typhimurium. In addition, we have shown that these compounds reduce virulence of the plant pathogen *Pseudomonas syringae* in a whole organism infection model. Finally, we have demonstrated that these compounds inhibit type III secretion as well as type II secretion-dependent functions and type IV pilus assembly, providing a proof-of-concept that inhibitors with broad-spectrum activity against Gram-negative secretion systems could potentially be developed to prevent and treat plant and animal bacterial diseases.

Plant Disease Management

The 2009 Tomato and Potato Late Blight Crisis: The Interaction of the Urban Home Garden and Commercial Agriculture—What Went Wrong and What We Learned

Overview and impact for extension, grower, and gardener
M. MCGRATH (1)
(1) Cornell University, Riverhead, NY, U.S.A.
Phytopathology 100:S161

The 2009 growing season was unprecedented for plant pathologists and anyone involved with producing tomato and potato in the northeastern U.S.A. For the first time late blight was found on tomato plants being sold in garden centers. Occurrence was widespread. Affected plants were not removed immediately as store managers do not have this authority under the consignment sale system and the pathogen is not regulated. Most gardeners had not seen late blight before and thus did not recognize it initially. Above average rainfall during June and July provided favorable conditions for pathogen spread from gardens with affected plants to other gardens and farms. Late blight occurs most years in the northeast, but mostly in the major potato production area of Maine. The 2009 late blight outbreak occurred much earlier and was more widespread than normal. The unusual source undermined the regional approach to management that is based on identifying the farm(s) where late blight starts to develop thus enabling other farmers locally and regionally to be alerted. These facts meant farmers initially were unprepared and many lost crops because late blight can be uncontrollable when fungicides are not applied preventively. Late blight outbreaks continued to occur during warm, low rainfall periods in Aug and in greenhouses in Oct. Many gardeners experienced for the first time arguably the most destructive plant disease, and learned they can have a devastating connection to agriculture.

Overview of the potted plant wholesale production business for the "Big Box" retail stores
B. D. OLSON (1)
(1) Dow AgroSciences, Geneva, NY, U.S.A.
Phytopathology 100:S161

The container plant business has provided customers with potted vegetable and flower plants for over 40 years. In the early 1900's the industry began as small local truck farms delivering field grown, bare-root, plants to the local retail businesses. Beginning in the 1950's, growers began growing potted plants in greenhouses and selling to farm supply stores and independent nurseries. Over time the industry has transformed dramatically to the present operations that deliver potted plants to the mega "big box" stores throughout the United States. The process of growing potted plants is a simple concept, but the operations are far from simple when supplying the quantity and quality of plants for today's retail giants. Tomato, pepper, and herb plants are started from seed. The seed is dropped by machine into plug trays. The trays vary in size from 100 cells to 500. Once the plugs have established a root system and reach transplant size, they are shipped to the growers for transplanting into larger containers. It then takes two to four weeks for the plants to reach a final size, at which point they are delivered to retail outlets for resale to home gardeners.

Perspective of the crisis from the state regulatory inspection service
S. KIM (1)
(1) Plant Disease Diagnostic Lab., Pennsylvania Department of Agriculture, Harrisburg, PA, U.S.A.
Phytopathology 100:S161

In 2009, late blight on tomato and potato plants appeared in June - earlier than in previous years - and was detected among samples from homeowners, private fresh-market tomato- and potato-farms, and big box retail markets. Under the Pennsylvania Plant Pest Act, the Pennsylvania Department of Agriculture (PDA) routinely inspects tomato plants at retail locations and production greenhouses. PDA inspectors issued Stop-Sale and treatment or destruct orders for any infected plants being offered for sale. PDA additionally inspects incoming out-of-state grown vegetable transplants each year. Between May-June, 2009, 6.5 million tomato transplants were inspected and found to be free of late blight symptoms. By September 2009, the Pennsylvania State University and PDA identified late blight in 58% of PA counties. The genotypes of *Phytophthora infestans* (US-8 >US-17>US-15 on tomato and US-8>US-14>US-7>US-10>US-17 on potato) that occurred between 1994 and 2004 have changed from predominantly resistant to mefonoxam-sensitive. Between 2004 and 2006, two genotypes were identified: US-14 (gpi 100/122; pep 100/100; A^2; mefonoxam-sensitive) on tomato and potato, and US-13 (gpi 100/100; pep 100/100; A^2; mefonoxam-sensitive) on tomato. In March 2010, PDA launched late blight targeted inspection services in response to the 2009 late blight outbreaks.

Science of the epidemic
K. DEAHL (1)
(1) USDA-ARS, Silver Spring, MD, U.S.A.
Phytopathology 100:S161

Late blight caused by Phytophthora infestans is one of the most destructive diseases of potato and tomato worldwide due to rapid asexual reproduction of the pathogen under conducive weather conditions. A total of 155 isolates of P. infestans were collected from the major crop production areas during 2007 to 2009 in the US. Isolates were characterized by their pathogenicity, mating type, and in vitro metalaxyl sensitivity. They were also subjected to molecular genotyping, by allozyme pattern, mitochondrial genomic haplotype, and DNA fingerprinting using the multilocus RFLP probe RG57. Before 2007, isolates collected from tomato and potato crops were mainly the US-8 or US-11 clonal lineage. However, P. infestans populations in the U.S.A. underwent a significant genetic shift in the 2007–2009 growth seasons; isolates with unique genotypes and epidemiological parameters including increased aggressive were detected in Florida and throughout the northeastern region of the United States. The greatest concern relating to the introduced new A1 + A2 populations was the potential impact of sexual recombination. Although sexual recombination was not yet detected, a decrease in the percentage of the US-8 clonal type implied that genomic diversity of the pathogen is changing quickly. Despite introduction of both mating types, in many regions new, but largely clonal populations have become established.

The 2009 potato and tomato late blight epidemics: Genealogical history, multiple sources and migration events
J. B. RISTAINO (1)
(1) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, U.S.A.
Phytopathology 100:S161

In 2009, potato and tomato late blight epidemics in the US were the worst in modern history due to a "perfect storm" of widespread inoculum distribution and conducive weather. Tomato late blight was found in the southeastern US from March to June and was found in the northeast in June on tomato transplants that were distributed through major garden center chains from MD to ME and Canada. Some of those tomato late blight strains also migrated to potato fields in several states. Prior to 2009, the US-20 and US-21 genotypes were common on tomato in Fl and NC. The US-8 genotype is still common on potato in NC and the NE but three new genotypes named US-22, US-23 and US-24 were also found on potato. The US-22 genotype (A2; Gpi 100/122) is the widespread strain that was found on tomato transplants. US-23 (A1, Gpi 100/100/111) also occurred on potato and tomato. We assessed the genealogical history of P. infestans genotypes from 2002–2009. Migration analysis suggested that gene flow occurred from tomato to potato in the eastern US populations of the pathogen. Isolates from a home garden in TN, commercial tomato fields in Long Island, NY in 2007 and FL in 2008 were also US-22. We used coalescent analysis and documented that the 2009 isolates were derived from the 2007 population. The data suggest that the US-22 genotype existed before the epidemics of 2009.

Policy meets practicality: Recommendations from an Extension Committee Task Force
M. A. DRAPER (1)
(1) USDA NIFA, Washington, DC, U.S.A.
Phytopathology 100:S161

In 2009, the losses from late blight reached the highest levels in over a decade. In response to this timely issue, several forums were held across the country to

identify needs and consider the developing issues and grower readiness in response to late blight. We have learned a great deal in recent years from experiences with outbreaks of several high consequence pests. The late blight epidemic provides one more cog in that gear. A common element among the best responses to a plant disease outbreak is the importance of preparation, a key to early detection and rapid response. Late blight is caused by a genetically complex pathogen with a challenging epidemiology. Many wide-area elements must be considered both tactically and strategically for a disease with the characteristics of late blight. Recognizing the significance of new disease records and developing a strategy for response, as well as determining the research and infrastructural needs to accomplish the expected response are critical pieces in a successful approach to recovery from a plant disease crisis. Feedback loops and strategies for future development of late blight resources from various producer and scientific forums will be discussed.

Biocontrol Beyond the Bench: Large-Scale, Successful Biocontrol

Challenges and successes of registering microbial biopesticides
M. BRAVERMAN (1), D. Kunkel (1)
(1) IR-4 Project, Rutgers University, Princeton, NJ, U.S.A.
Phytopathology 100:S162

The IR-4 Project has had a regulatory assistance program since 1982 whereby we help public sector researchers and small businesses in the EPA registration process. The IR-4 Project also has been funding researchers through a small efficacy grant program since 1995. Grants are divided into 3 stages which are early stage projects on products in which no toxicology work has been preformed, advanced stage projects which involve registered products looking to expand the labeled uses and demonstration stage projects which are for on-farm demonstration trials of registered uses. The demonstration stage program is co-funded and selected in cooperation with the Biopesticides and Pollution Prevention Division of EPA. Some of the registrations IR-4 has assisted on have included Aspergillus flavus AF36 for aflatoxin management in cotton, pistachio and corn, Verticillium WCS 850 for Dutch elm disease and bacteriophage for management of Xathamonas and Pseudomonas. Registration under way include Trichoderma hamatum isolate 382, a viral coat protein for the management of plum pox, and tobacco mild green mosaic tobamovirus for management of the invasive weed, tropical soda apple. The primary focus has been on U.S. registrations but cooperative work with Canada and African countries has also developed.

Bringing a broad spectrum bioherbicide to market
A. WATSON (1)
(1) McGill University, Ste-Anne-de-Bellevue, QC, CANADA
Phytopathology 100:S162

There have been many bioherbicide research projects, but very few have become commercial products. Dandelion is an important weed in North America and represents one of the single largest target pests for application of pesticides in North America. Chemical control using 2,4-D is the accepted method of dandelion control but public awareness and concern about the potentially harmful effects of lawn care chemicals lead provincial and municipal governments in Canada to ban the use of pesticides on lawns necessitating the discovery and development of alternative weed control strategies. Now there is an effective biological option. SARRITOR is the first bioherbicide developed and registered in Canada for control of dandelion and other broadleaf weeds in turfgrass. The active ingredient of SARRITOR is a naturally occurring broad host range fungal plant pathogen, Sclerotinia minor (isolate IMI 344141). Commercialization of SARRITOR has been the culmination of combined efforts and innovation stemming from McGill University's weed research laboratory in partnership with McGill's Technology Transfer Office, Natural Sciences and Engineering Research Council of Canada, government ministries and laboratories, universities, and industry collaborators. SARRITOR commercial was launched in 2008 while SARRITOR domestic was launched in 2010. U.S. registration is pending.

Understanding your customer and delivering a quality product
B. FOSTER (1)
(1) BioWorks, Victor, NY, U.S.A.
Phytopathology 100:S162

While some believe a patented organism displaying biocontrol activity is the final step to the successful commercialization of a new product, in reality it is only the first step. The goal of a novel biocontrol product is no different than a novel new synthetic active ingredient - to provide a unique, differentiable solution that addresses specific, unmet customer problems or needs. The features, advantages and benefits of the novel biocontrol product must be compelling enough for the customer to switch from what they are currently using to the novel biocontrol product. This presentation will address some of the "normal" commercialization issues associated with introducing a new biocontrol product in to the agricultural market.

Lockdown: Collego bioherbicide gets a second act
K. CARTWRIGHT (1), D. Boyette (2), M. Roberts (3)
(1) ARI, Inc., Fayetteville, AR, U.S.A.; (2) Southern Weed Science Research Unit, Stoneville, MS, U.S.A.; (3) Natural Industries, Houston, TX, U.S.A.
Phytopathology 100:S162

Collego™, originally registered with the EPA in 1982, was one of the first two commercially available mycoherbicides. The product was used successfully to control Northern jointvetch (*Aeschynomene virginica*) in mid-south rice for some 20 years. About 10 years ago, however, the mycoherbicide was discontinued per the close of the marketing company and, as a result, the product lost EPA registration status. In 2005–2006, Agricultural Research Initiatives (ARI, Inc.-Fayetteville, Arkansas) pursued successful EPA registration and has been awarded three section 3 (C) registrations for the active ingredient (*Colletotrichum gloeosporioides* f. sp. *aeschynomene*) under the trade name LockDown™ with three formulations applicable (retro, XL and Liquid). Working with USDA and University scientists, extension personnel, consultants and farmers, successful field and greenhouse trials were completed in 2006 and 2007. Subsequently, manufacturing was scaled up and optimized in 2008 with product launch during the summer, 2008. In 2009, Natural Industries (Houston, TX) began producing and marketing the LockDown product. This year (2010) represents the third full year of market. To date, production, quality control and overall efficacy for the product has been excellent. In the future, we anticipate sales growth through more aggressive marketing strategies and penetration into additional timing and management slots for use of the product in rice.

Working together: Partnering with grower organizations from development through distribution to make Aflatoxin Biocontrol a reality in the US/Africa
P. J. COTTY (1), R. Bandyopadhyay (2)
(1) USDA/ARS, Department of Plant Pathology, University of Arizona, Tucson, AZ, U.S.A.; (2) IITA, Ibadan, NIGERIA
Phytopathology 100:S162

Biocontrol products directed at competitive exclusion of aflatoxin producers by atoxigenic strains of *A. flavus* are the only technologies registered for pre-harvest mitigation of aflatoxin contamination. These products provide useful levels of efficacy both during crop development and postharvest. Lessons learned with atoxigenic strains shed light on models through which public sector organizations can partner with grower organizations to develop and distribute biocontrol agents. In Arizona, cotton industry organizations partnered with the USDA to field test, register, and manufacture the biocontrol agent AF36. This experience was built upon by IITA in development and registration of atoxigenic strains in partnership with public/private and governmental organizations in Nigeria. Major obstacles to success of such partnerships include requirements for initial capital outlay and technical requirements imposed by the pesticide registration process. Farmers readily accept aflatoxin biocontrol based on both experimental data and personal experience with efficacy. However, products still need marketing and distribution systems. In Africa, unlike the U.S. where market forces to meet aflatoxin standards push adaptation, commercial incentives will combine with government responsibility for public health to drive demand for biocontrol. Several models for development and deployment are needed for biocontrol to meet worldwide needs for aflatoxin management.

Creating Possibilities for Sustainable Postharvest Disease Control Through Integrated Approaches to Both Pre- and Postharvest Fungicide Resistance Management

Resistance mechanisms in postharvest fungicides
U. GISI (1), H. Sierotzki (2)
(1) University of Basel and Syngenta Crop Protection, Stein, SWITZERLAND; (2) Syngenta Crop Protection, Stein, SWITZERLAND
Phytopathology 100:S162

The most important pathogens attacking fruits like citrus, apples, peaches and potato tubers after harvest include several species of *Penicillium*, *Botrytis*, *Glomerella*, *Monilinia*, *Alternaria*, *Fusarium* and *Helminthosporium.* A range of fungicide groups are used to control these pathogens such as phenyl-pyrroles (PPs, fludioxonil), anilinopyrimidines (APs, e.g. pyrimethanil), quinone outside inhibitors (QoIs, e.g. pyraclostrobin, azoxystrobin), and sterol biosynthesis inhibitors (SBIs, e.g. fenhexamid, several azoles). As with older fungicides such as benzimidazoles (MBCs, e.g. thiophanate-methyl), resistance can evolve after intensive use and limit pathogen control to a certain degree. The extent and level of resistance evolution can be assessed using a range of elements including intrinsic and extrinsic fungicide risks, as well as pathogen and agronomic risks. Risk factors are assessed based on molecular, genetic, biochemical, physiological and population aspects. Resistance can be monogenic as in MBCs and QoIs or polygenic as in DMIs. For PPs, resistance is claimed to be linked to os-2 and ABC transporter genes, for APs by two mutations in the cystathionine-γ-synthase (cgs) gene. Resistance management should include all available elements including good agronomical practice, limitation of inoculum carry-over from field to packing house, reduction in the number of fungicide treatments and use of mixtures and alternations of fungicides with different modes of action.

Postharvest resistance management: An integration of strategies encompassing the pathogen, fungicide properties, and epidemiological principles
H. FORSTER (1), J. Adaskaveg (1)
(1) University of California, Riverside, CA, U.S.A.
Phytopathology 100:S163

Historically, resistance to postharvest fungicides has developed in pathogens of fruit commodities that are stored for extended periods (e.g., citrus and pome fruits). Strategies to minimize the selection for resistance begin with simultaneous registrations of compounds with different modes of action (MOA) that are highly effective in reducing decay (prevent selection) and suppress sporulation of pathogens (prevent wild-type population displacement). Resistance management must prevent repeated exposure of high pathogen populations to any fungicide. Ideally, rotations or mixtures of fungicides with different MOAs reduce resistance potential because a lower resistance frequency exists to multiple sites of action. Packinghouse practices that increase the likelihood of resistance development include all methods that lead to sub-optimal fungicide coverage, decreased fungicide residues, or reduced fruit quality. Important chemical properties include systemic activity, persistence, temperature and pH stability, compatibility with fruit coatings and oxidizing sanitizers, or any property that facilitates integrated management strategies. Sequential applications of fungicides as aqueous treatments for decay control and in fruit coatings for sporulation control are ways to optimize performance in a single process. Sanitation practices are essential to minimize pathogen survivors and reduce the total population of pathogens that are exposed to a fungicide.

Management of fungicide resistance in postharvest pathogens of pome fruits: Integrated approaches from orchard to storage
C. XIAO (1), Y. Kim (1), Y. Yin (1)
(1) Washington State University, TFREC, Wenatchee, WA, U.S.A.
Phytopathology 100:S163

Recently registered pre- and postharvest fungicides pyraclostrobin+boscalid, fenhexamid, fludioxonil, and pyrimethanil have provided new tools for control of postharvest diseases in pome fruits. In the meantime, postharvest pathogens *Penicillium expansum* and *Botrytis cinerea* are high-risk pathogens for the development of fungicide resistance. Baseline sensitivities and resistance-monitoring programs for key fungicides have been established. Dual resistance to pyraclostrobin and boscalid had developed in *B. cinerea* in apple orchards where the fungicides had been used. Resistance of *P. expansum* to pyrimethanil was first detected in 2009 from decayed apple fruit. While these fungicides are being increasingly used by the fruit industry, strategies for management of fungicide resistance in these pathogens need to be developed and implemented. Management of fungicide resistance in postharvest pathogens should involve both orchard and packinghouse practices. Critical factors to be considered include disease cycles from orchard to storage under different postharvest handling systems, inherent risk of both fungicides and pathogens for resistance development, biological and ecological characteristics of fungicide-resistance phenotypes, patterns of cross resistance to other fungicides. Integrated approaches involving practices from orchard to storage for management of fungicide resistance in postharvest pathogens as well as for postharvest disease control will be discussed.

Resistance management strategies for new postharvest fungicides - Pace International perspective
P. G. SANDERSON (1), D. Bylemans (2)
(1) Pace International, LLC, East Wenatchee, WA, U.S.A.; (2) Janssen Pharmaceutica N.V., Beerse, BELGIUM
Phytopathology 100:S163

In 2004, two new fungicides, Penbotec (pyrimethanil) and Scholar (fludioxonil) were registered in the U.S. for use on pome fruit. These were the first new classes of chemical fungicides to gain postharvest registrations since Benlate was registered in the early 1970's. By 1978, it was reported that about 30% of isolates of *Penicillium expansum* (causal agent of blue mold) recovered from packing houses were resistant to benzimidazole fungicides. This level of resistance has persisted until the recent introduction of these new chemicals. Hence, we have a good the model for fungicide resistance development within pome fruit postharvest systems. In conventional packing of pome fruit, fungicides may be applied either at harvest as a drench, or as line sprays during packing. Studies on growth and dispersal of populations of *P. expansum* clearly showed that field bins were the reservoirs of benzimidazole resistant populations, and that drenching practices were responsible for the accumulation and dispersal of those resistant populations. Although these new fungicides are considered to have a somewhat reduced potential for development of resistance compared to the benzimidazoles, we can expect a similar loss of effectiveness unless changes are made in fruit and equipment handling. The effects of fungicide alternation schemes, advances in sanitation methods, and the effect of new application technologies on resistance development will be explored.

Resistance management strategies for new postharvest fungicides - Syngenta perspective
A. COCHRAN (1), E. Tedford (1), D. McKenzie (2)
(1) Syngenta Crop Protection, Greensboro, NC, U.S.A.; (2) Syngenta AG, Basel, SWITZERLAND
Phytopathology 100:S163

Postharvest decay is one of the greatest challenges facing the fruit and vegetable industries worldwide. Fungicides may be used to control post harvest decays but their management is key, as continuous and exclusive use may lead to development of resistance. Fungicide resistance management for postharvest decay is challenging as some fruits and vegetables have to be stored for a long time and/or transported long distances. There are many factors that contribute to resistance development, including exclusive use of fungicides having one mode of action, highly fecund pathogens such as Penicillium spp., and storage conditions that are ideal for pathogen infection and spread. Common strategies to offset resistance development in postharvest pathogens include the use of sanitation, alternation of fungicides having different modes of action, and/or the use of fungicide pre-mixtures. In an effort to better manage fungicide resistance to storage diseases Syngenta is combining good sanitation practices with the use of fungicide pre-mixtures in fruit and vegetables. This pre-mixture approach is currently being employed by Syngenta for citrus fruit and is being developed for other fruits and vegetables. These mixtures represent a new and innovative approach to postharvest disease control aimed at providing broad-spectrum decay control built on a sound resistance management strategy when combined with proper disease management in the field and use of postharvest sanitation when possible.

Edible and Medicinal Mushrooms: Diversity, Commercial Production, and Disease Management in High-Volume Production Facilities

Global expansion in gourmet and medicinal mushroom cultivation and use
M. WACH (1)
(1) Sylvan Inc., Kittanning, PA, U.S.A.
Phytopathology 100:S163

Mushrooms have been held in fascination by humans dating back at least to the era of the Caveman. Whether they were consumed as food, utilized as part of spiritual ritual or used as a poison, these higher basidomycetes play a significant role in the evolution of mankind. In China, as early as 1245 A.D., Chen Yen-yu published details on the development, morphology, growing methods and preparation of 15 different varieties of mushrooms. Commercial cultivation can be traced back at least as far as 1313, when Wang Zeng described the growing of Shiitake (*Lentinula edodes*). Cultivation of the button mushroom *Agaricus bisporus*, was first described by Tournefort in 1707, wherein he describes the use of spent horse manure on which mushrooms were growing for use as inoculum, facilitating continuous production and spawning the modern mushroom industry. Today, some 14,000 mushroom species have been reported, of which about 60 have been

commercially cultivated. While the majority of these cultivated species are utilized as a food, a growing number of edible fungi are finding utility in other ways. This presentation will explore the growth and development of the commercial mushroom industry including a discussion of culinary, medicinal and other uses.

Disease management in commercial mushroom facilities: Controlling the fungus' fungus

D. BEYER (1)
(1) The Pennsylvania State University, State College, PA, U.S.A.
Phytopathology 100:S164

Mushroom cultivation is an ideal model of the disease triangle, pathogen-host-environment interaction. Agaricus bisporus, the white and brown commercially grown mushroom is exposed to many different types of pathogens during its cropping cycle, insects, bacteria, nematodes viruses and other fungi. Controlling these pathogens requires a solid integrated pest management program and a good understanding of the biology for pests and diseases. Better and safer methods must be found as alternatives to chemicals and towards the environmental and cultural manipulation without lowering yield or quality of mushrooms. This part of the symposium will describe the disease management strategies used in commercial mushroom farms. Several major fungal pathogens, their signs and symptoms will be described and how cultural management and to a lesser extend chemicals are used to manage these diseases.

Developing mushroom cultivation curricula at the university

M. DAVIS (1)
(1) University of California, Davis, Davis, CA, U.S.A.
Phytopathology 100:S164

A course on the cultivation of edible mushrooms is a marvelous way to connect students from all academic backgrounds with both biology and agriculture. The annual undergraduate course 'Mushroom Cultivation' at UC Davis is extremely popular and always has a waiting list to register. The 11-week course introduces methods of growing edible mushrooms, including culture maintenance, basic mushroom substrate preparation, composting, spawn generation techniques, inoculation methods, harvesting, and pests and pest management. The students grow Oyster, Shiitake, Button, Lion's mane, and Reishi mushrooms. A field trip to a local mushroom farm is often included in the curriculum. The history of mushroom production and recent trends in the diversification of edible mushrooms are discussed. At least one biology prerequisite course is recommended but is not required. The goals of the course are to provide the students with an understanding of the vocabulary used in the mushroom industry, the experience of growing mushrooms, an understanding of the biology of fungi, and hopefully, a lifelong interest in mushrooms. An appreciation for agriculture is one of the underlying premises of the course.

Certifying mushrooms organic to the USDA National Organic Program Standard

T. ELLOR (1)
(1) Phillips Mushroom Farms, Kennett Square, PA, U.S.A.
Phytopathology 100:S164

Certifying and growing mushrooms to the USDA National Organic Program (NOP) crop standard is challenging on a number of fronts. There is no part of the NOP standard that applies specifically to mushrooms, so applying a standard that was written predominantly for field crops, orchards, and produce grown in soil outside presents unique difficulties. Growing organic mushrooms in the 'Mushroom Capitol of the World' AKA the 'Mushroom Disease Capitol of the World' presents another set of problems. Familiarity with the Organic Standards and how and where it does and does not apply to mushrooms is critical to writing an effective organic system plan. The 2nd set of problems can be approached by being familiar with pests and diseases that affect mushroom production, excellent sanitation and management, and sound IPM practices which include alternatives to conventional controls.

Identifying Quantitative Resistance Using Modern Technologies—Challenges for Plant Breeding

Bioinformatic strategies for predicting candidate genes under disease resistance QTL

R. M. DAVIDSON (1), P. A. Reeves (2), P. M. Manosalva (3), J. E. Leach (4)
(1) Department of Plant Biology, Michigan State University, Fort Collins, CO, MI, U.S.A.; (2) USDA, National Center for Genetic Resources Preservation, Fort Collins, CO, U.S.A.; (3) Boyce Thompson Institute for Plant Research, Ithaca, NY, U.S.A.; (4) Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 100:S164

Recent advances in genome sequencing have resulted in a plethora of genetic, genomic and transcriptomic data in many online databases. We have undertaken a comprehensive study aimed at combining numerous sequence and expression datasets of the large multi-member germin protein family that is known to include genes involved with broad-spectrum disease resistance. First, we examine phylogenetic relationships and functional diversity of germins across diverse genera including monocots and dicots, and then focus on rice (*Oryza sativa*) as a model. The comparative analyses allow us to predict candidate germin gene lineages with possible relevance to disease resistance across taxa. Comprehensive data for rice, including a well-annotated genome sequence, robust genome-wide expression data and published QTL data provide us the platform to identify candidates at the gene level. The use of bioinformatics to layer data types, detect candidates and connect them across plant species will become more powerful as an increasing number of crop genomes are sequenced and gene functions are determined. This approach will expedite the prediction of genes underlying QTL that are useful for crop improvement.

QTL use for development of host resistance and putting it to use-Industry perspective

G. TABOR (1)
(1) Pioneer Hi-Bred, Johnston, IA, U.S.A.
Phytopathology 100:S164

The availability and affordability of high throughput molecular marker genotyping has greatly facilitated the identification and utilization of disease Quantitative Trait Loci (QTL) in commercial breeding. QTL-based marker assisted selection has reduced cost and time needed for development of disease-resistant varieties in many crops. A disease QTL has to meet several criteria to be commercially useful and these criteria vary according to the crop and disease in question. In general, a commercially viable disease QTL should not have adverse effect, due to either pleiotrophy or tight linkage, on critical commercial traits. Some disease QTL are of high effect and/or probably encompass major gene(s) for disease resistance; these QTL tend to have higher chance of being utilized in commercial breeding. Conversely, many QTL are of minor effects and some of them may not be incorporated in commercial products due to many considerations including cost. With the advancement in genomics, high-throughput genotyping technology and supporting technologies, one of the limiting steps in QTL identification has become lack of reliable phenotypic assays. Disease phenotypic assays have not advanced in parallel with genotyping technology. Limited research emphasis on understanding the basic biology, epidemiology and genetics of host-pathogen interaction for most pathosystems have limited advancements in development of new phenotypic assays.

Proteomics in identifying potential markers for developing broad spectrum resistance

Z. CHEN (1), S. Park (1), C. Zhang (2)
(1) Louisiana State University, Baton Rouge, LA, U.S.A.; (2) Ames, IA, U.S.A.
Phytopathology 100:S164

Soybean rust, caused by *Phakopsora pachyrhizi*, was first reported in the continental U.S. in late 2004. Since then it has been spreading steadily north in the past years. Severely infected soybean plants can be quickly defoliated and resulted in significant yield losses of up to 80%. There is no resistant soybean cultivar yet available to growers. Recently, soybean lines with single-gene resistance (such as *Rpp1-5*) were identified. However, these lines showed resistance to limited numbers of rust races and became ineffective soon after they were identified. In an effort to identify potential markers for developing durable broad-spectrum resistance to soybean rust disease, a proteomics approach was used to compare protein profile differences during compatible and incompatible interactions between soybean and *P. pachyrhizi*. Differentially expressed proteins were identified and sequenced through tandem mass spectrometry. The expressions of some of the proteins were further examined during the time course of rust infection to determine how early host responds to pathogen attack and how their expressions are changed at different infection stages. Two of the infection-induced proteins, pathogenesis-related protein 10 and chalcone isomerase 1, which were upregulated during early and middle stages of infection, respectively, were further investigated through VIGS to determine their roles in soybean resistance to rust disease.

Using network biology to identify quantitative genetic variation altering signaling in both plant host and generalist pathogens
D. J. KLIEBENSTEIN (1)
(1) University of California, Davis, Davis, CA, U.S.A.
Phytopathology 100:S165

Botrytis cinerea is a highly diverse pathogen with natural variation modulating an extreme range of phenotypes that is central to being a generalist pathogen. We are combining modern systems biology with natural variation in *Botrytis* and *Arabidopsis* and Tomato to identify how the pathogen and plant genetic variation interact. This is highlighting fundamental mechanisms influencing broad host resistance. By investigating quantitative trait loci that control differential resistance to *Botrytis* isolates we can link global variation in plant defense gene and defense metabolite expression with altered Botrytis virulence. This is showing that jasmonic acid is only required for resistance to a subset of *Botrytis* genotypes. Understanding what is conserved and variable within a generalist pathogen identifies targets that will not overcome by pathogen genetic variation. One complexity to systems biology is the concept that only genomics experiments done in the presence of the pathogen will be informative. Given the diversity within these pathogens this supposition creates massively expensive experiments. We are testing the concept that systems biological approaches can identify predictive signatures of generalist resistance in the absence of the pathogen itself. We will present evidence that we can identify mechanisms controlling pathogen resistance without conducting the experiments in the presence of the pathogen.

Induced Resistance: Where Does This Fit in IPM Programs

Induced systemic resistance by biological control agents
P. BAKKER (1)
(1) University of Utrecht, Utrecht, NETHERLANDS
Phytopathology 100:S165

The mechanisms that underlie biological control of plant diseases using antagonistic microorganisms include competition for space and nutrients, antibiosis, lytic activity, and induced systemic resistance (ISR). In the last decades it has become clear that elicitation of ISR by biological control agents is a widespread phenomenon. Many microorganisms that were previously reported to suppress disease by competition, antibiosis or lytic activity were also demonstrated to elicit ISR. Induced resistance is a state of enhanced defensive capacity developed by a plant reacting to specific biotic or chemical stimuli. ISR is typically studied in systems in which the biocontrol agent and the pathogen are inoculated and remain spatially separated. Much progress has been made in elucidating the signal transduction pathways in the plants involved in ISR and the microbial elicitors of ISR. The diversity of microorganisms, plant species, signal transduction pathways and microbial elicitors in relation to induced resistance, and ways to improve the efficacy of ISR will be discussed.

Seed or soil applied bacteria that induce resistance-Use in IPM programs
J. W. KLOEPPER (1)
(1) Auburn University, Auburn, AL, U.S.A.
Phytopathology 100:S165

Studies with many different bacterial biocontrol agents, including PGPR, have demonstrated that one mechanism by which effective strains reduce plant disease is elicitation of induced systemic resistance (ISR) in various model systems. Applications of these findings in practical disease management strategies for commercial agriculture are now possible using seed treatment with aerobic spore-forming PGPR (the bacilli). A case study is *Bacillus pumilus* strain INR-7 which was isolated from inside surviving cucumber plants in a field with a high incidence of cucurbit wilt disease caused by *Erwinia tracheiphila*. Subsequent research revealed that the strain elicited ISR in cucumber against multiple pathogens in the field and reduced the feeding preference of cucumber beetles, which was related to decreased plant concentrations of cucurbitacin, a feeding cue for the beetles. In soybean, strain INR-7 elicited increased plant cell wall lignification which was associated with biocontrol. Strain INR-7 is registered by the EPA as a biological fungicide and which is currently marketed as Yield Shield™ by Bayer Crop Sciences for managing *Fusarium* spp. and *Rhizoctonia solani* on soybean and beans. While we have an understanding of some plant biochemical changes that occur during the signal transduction elicited during ISR by INR-7, we do not yet know what component of the strain triggers ISR. Future directions with integrated biocontrol strategies will be discussed.

Foliar applied Bacillus that induce resistance-Use in IPM Programs
B. J. JACOBSEN (1)
(1) Montana State University, Bozeman, MT, U.S.A.
Phytopathology 100:S165

Several foliar applied Bacillus-based biological control agents (BCAs) are registered by the U.S. EPA for disease control. *Bacillus subtilis* isolate QST 713, *B. mojaviensis* isolate 203-7, and *B. mycoides* isolate BmJ have been reported to effect disease control involving induced resistance. The BmJ isolate provides control of a wide range of diseases caused by fungal, bacterial and viral pathogens by inducing systemic resistance in plants. Induction is salicylic acid independent and involves signaling through the NPR1 and ethylene pathways. Induced resistance has been demonstrated in sugarbeet, cucumber, potato and tomato. When using induced resistance applications must be made before infection for optimal results. Control using BmJ alone is in the 30–90% range and in most pathosystems tested, inferior to the best commercial fungicides. However, when used with host resistance, reduced rates or applications of fungicides control is often equal to the best labeled fungicides. Research on Cercospora leaf spot of sugarbeet (*Cercospora beticola*), showed incorporation of BmJ in fungicide control programs resulted in reduced fungicide resistance. *Bacillus* BCAs such as BmJ offer the opportunity to control bacterial and viral pathogens for which alternatives are only equally effective or are not available. For fungal pathogens, this BCA offers an alternative in fungicide resistance management and alternatives for both conventional and organic growers. BmJ is compatible with a wide range of pesticides.

The role of *Trichoderma* in crop management systems
G. HARMAN (1), F. Mastouri (1)
(1) Cornell University, Geneva, NY, U.S.A.
Phytopathology 100:S165

Trichoderma spp. have been know for decades as biocontrol fungi. However some strains are endophytic plant symbionts. They invade and colonize roots, thereby inducing plant resistance, which results in localization of the fungi. Some strains also can invade and colonize twigs and stems. Successful use of Trichoderma requires that highly efficient strains be discovered or produced. Earlier, we believed that antibiosis and mycoparasitism were principal mechanisms of biocontrol, but we now know that induced systemic resistance is probably more important. However, biocontrol is only a subset of the advantages that effective endophytic strains confer. They also induce resistance to a variety of abiotic stresses, including water deficit, temperature, salt and osmotic stress. They also increase nitrogen use efficiency (NUE) in plants; it is anticipated that we can reduce nitrogen use for selected crops by 30% without reducing yields. These applications have major implications for plant agriculture. NUE can, for example, reduce air and water pollution from agriculture and can improve food security for small holders who cannot afford sufficient nitrogen fertilizer to obtain maximum yields of plants. The fungi create these numerous benefits because they alter plant gene expression. Photosynthetic efficiency, especially in the presence of stresses such as drought, is improved, as is plant ability to ameliorate effects of toxic reactive oxygen species.

Chemical compounds that induce resistance-Use in IPM programs
A. H. TALLY (1)
(1) Syngenta Crop Protection, Greensboro, NC, U.S.A.
Phytopathology 100:S165

Disease protection through induced resistance has been known for many years, but it was not until the development of acibenzolar-S-methyl (ASM/Actigard/Bion) that researchers had a non-biological tool that induced the plant response similarly to biological induction. Other substances are also known to have similar effects – INA, salicylic acid, laminarin, harpin - although the mechanisms may be different. What is evident, with a few exceptions, is that the level of disease protection is more suppression of disease rather than excellent control that is provided by many of the traditional fungicides. Compounds such as ASM are useful tools as there are no alternatives for some diseases, and in many cases, the compounds improve performance of the fungicide and provide plant health benefits which make them an excellent fit in IPM programs. Depending on the crop, these products can suppress a broad spectrum of pathogens as well as provide another tool for resistance management. There is also evidence that Actigard can help maintain the utility of resistant cultivars longer. Rate ranges and use patterns are being studied more to be able to more fully realize the additional benefits in an IPM program.

Kasugamycin: The Risks and Benefits of Introducing a New Antibiotic

Re-discovery of kasugamycin for managing fire blight and other bacterial diseases of plants in the United States
J. ADASKAVEG (1), H. Forster (2), L. Wade (3)
(1) University of California, Riverside, CA, U.S.A.; (2) University of California, Davis, CA, U.S.A.; (3) Roseville, CA, U.S.A.
Phytopathology 100:S166

Kasugamycin was first discovered and produced as a commercial fermentation product of *Streptomyces kasugaensis* in Japan in the 1960s. In 2006, after the US-EPA allowed import tolerances on food commodities, we requested a U.S. registration through the federal IR-4 program for managing fire blight, walnut blight, and other bacterial diseases (e.g., bacterial canker). Although classified as a systemic aminoglycoside antibiotic, kasugamycin is an antimicrobial with a unique mode of action from antibiotics, has fungicidal/bactericidal activity, and is not used in animal or human medicine. The proposed registration is based on a need for new compounds for managing bacterial plant diseases. Streptomycin-resistant strains of the fire blight pathogen *Erwinia amylovora* are widespread in the U.S. and recently, strains less sensitive to oxytetracycline were detected and associated with crop losses in CA. Copper-resistant strains of the walnut blight pathogen *Xanthomonas juglandis* are also widespread in CA. These pathogens are sensitive to kasugamycin and sensitivity baselines were developed. Because of the potential of resistance developing in microbes to any single-site mode of action material, disease management studies using rotations and mixtures of kasugamycin with antibiotics, biologicals, and broad-spectrum compounds (e.g., copper, mancozeb, captan) were conducted, shown effective, and are suggested with the introduction of kasugamycin.

Kasugamycin – A novel antibiotic for North American agriculture
V. J. SPADAFORA (1), G. Orr (1), L. Wade (1), M. Wiglesworth (1)
(1) Arysta LifeScience North America, LLC, Cary, NC, U.S.A.
Phytopathology 100:S166

Kasugamycin (KM) is a novel aminoglycoside antibiotic being developed by Arysta LifeScience in the U.S.A. and Canada, in conjunction with Hokko Chemical Industry and leading research institutions under the trademark Kasumin®. New antibiotics, while effective, have not been registered due to concerns that resistance developed in populations of plant pathogens will be transferred to human and animal pathogens. The lack of new agricultural antibiotics has limited options for bacterial disease control in plants has increased selection pressure and the incidence of resistance to existing materials. Kasumin is an effective, useful new tool for North American Agriculture that carries minimal intrinsic risks for use in plant protection and to human and veterinary medicine. KM is relatively inactive against human and veterinary pathogens and is not used for these purposes. It's site of action within the bacterial protein synthesis pathway is different from that of other aminoglycoside antibiotics, allowing KM to be effective against strains of plant pathogens that are resistant to other antibiotics, making it an effective resistance management tool. The efficacy of KM has been proven globally, and it is sold in 30 countries since it's introduction in 1966. In North America, efficacy has been proven in numerous research trials, under emergency use exemptions, and under commercial conditions in Michigan and Mexico. ®Kasumin is a registered trademark of Hokko Chemical Industry.

EPA approaches for evaluating antibiotic uses in the context of FIFRA
L. ROSSI (1)
(1) EPA, Crystal City, VA, U.S.A.
Phytopathology 100:S166

EPA's risk assessment approaches in the consideration of new requested uses of antibiotic materials under FIFRA will be discussed. The components of the EPA's safety analysis, which includes food and drinking water exposures, MRL establishment, as well as evaluations for worker exposure and risk, and ecological effects will be presented. EPA assessments relative to evaluating the safety of new drugs from the standpoint of resistant bacteria will be covered. In this regard, EPA is using an approach similar to that of FDA to characterize the safety of antimicrobial drugs, which will be described. Milestones around the public participation process EPA applies to first domestic food uses will be explained as well as plans for direct dialogue and coordination with partner agencies, such as FDA and CDC. Regulatory mechanisms that may be available through FIFRA labeling to enhance the safety and utility of antibiotics used in agriculture will be discussed. In addition, post registration considerations such as monitoring for the development of resistant bacteria will be summarized for discussion.

Kasumin: Field results for fire blight management and evaluation of the potential for resistance development in *Erwinia amylovora*
G. W. SUNDIN (1), G. C. McGhee (1)
(1) Michigan State University, East Lansing, MI, U.S.A.
Phytopathology 100:S166

The antibiotic Kasumin (Arysta Corp.; Cary, NC) was evaluated under field conditions for control of the blossom blight phase of fire blight, caused by *Erwinia amylovora*. Orchard studies were conducted on the highly-susceptible varieties 'Gala' and 'Jonathan', and the trees were inoculated with a concentrated suspension (1×10^6 cfu/ml) of the virulent strain *E. amylovora* Ea110. Incidence of fire blight disease, as measured by the occurrence of blossom blight symptoms in inoculated plots, was relatively high in nontreated control trees in all six experiments conducted between 2006 and 2009, ranging from 35.6 to 72.5 percent infection. Application of Kasumin to trees at least 24 h prior to and at least 24 h following inoculation with strain Ea110 resulted in high levels of disease control that were not significantly different from the standard streptomycin treatment in any experiment. We assessed both the potential development of spontaneous mutants of *E. amylovora* with kasugamycin (Ks) resistance and the possibility of acquisition of a transferrable Ks-resistance gene(s). Spontaneous Ks resistance was only detected when *E. amylovora* strains were incubated in broth medium containing progressively larger concentrations of Ks. These mutants harbored mutations in the *ksgA* gene, and most were reduced in virulence in an immature pear assay. We are currently attempting to isolate Ks-resistance genes from the nontarget microflora present in apple trees and orchard soil.

Resistance management strategies for bacterial pathogens: What works?
K. B. JOHNSON (1)
(1) Dept. Botany and Plant Pathology, Oregon Sate University, Corvallis, OR, U.S.A.
Phytopathology 100:S166

With fungicides, proven strategies to slow development of resistance in the target pathogen include utilization of appropriate material dose, of mixture and/or rotation partners, and of limits to material use. With bacterial plant pathogens, documented successes in resistance management are rarer, perhaps because bacteria more readily acquire resistance and/or because there are so few effective bactericides. Using Kasumin as a case study, potential resistance management strategies for the fire blight pathogen, *Erwinia amylovora*, will be deliberated and contrasted with other bactericides used for fire blight control. Data demonstrating effective fire blight suppression with mixtures of Kasumin and oxytetracycline, and with Kasumin integrated with biological control will be presented. These resistance management strategies will be discussed in relation to the mechanisms and heritability of resistance, to the potential costs of resistance to reproductive fitness, and to the degree of selection pressure imposed by the bactericide treatment.

Restoring Forest Ecosystems Impacted by Invasive Pathogens

Can whitebark pine be saved?
E. M. GOHEEN (1), J. Schwandt (2)
(1) USDA Forest Service, Forest Health Protection, Central Point, OR, U.S.A.; (2) USDA Forest Service, Forest Health Protection, Couer d' Alene, ID, U.S.A.
Phytopathology 100:S166

Whitebark pine (*Pinus albicaulis*), a five-needled pine of mountainous regions in western North America, is considered a keystone species in the fragile high elevation ecosystems it inhabits. The future of whitebark pine is of substantial concern due to the species acute vulnerability to the non-native fungus *Cronartium ribicola* (cause of white pine blister rust), its susceptibility to infestation by mountain pine beetle (*Dendroctonus ponderosae*) which may kill trees that harbor natural resistance to blister rust, its risk of being destroyed in large and intense wildfires, its likelihood of being replaced by fire intolerant species due to fire exclusion, and the potential impacts of warming temperatures at high elevations. Implementation of a conservation and restoration program to protect and enhance existing populations, provide regeneration opportunities, and increase the proportion of trees with natural resistance to white pine blister rust can reverse this trend. Restoration projects underway include: rust surveys and monitoring to determine host status, collecting and storing whitebark pine seed, identifying and testing trees for natural resistance to white pine blister rust, planting blister rust-resistant seed

or seedlings, using silvicultural methods to reduce competing vegetation and create planting sites, encouraging natural regeneration, and treating blister rust-resistant trees to prevent beetle attacks.

Management of Port-Orford-cedar (*Chamaecyparis lawsoniana*) in the presence of the non-native pathogen *Phytophthora lateralis*
R. A. SNIEZKO (1), D. J. Goheen (2)
(1) USDA Forest Service, Cottage Grove, OR, U.S.A.; (2) USDA Forest Service, Central Point, OR, U.S.A.
Phytopathology 100:S167

Port-Orford-cedar (POC), a unique and valuable tree native to SW Oregon and NW California, is affected by a virulent, non-native pathogen, *Phytophthora lateralis*. Infection results in death of hosts of all ages. The goal of the POC management by the Forest Service and BLM is to maintain POC as an ecologically and economically significant species on federal lands. The integrated strategy developed seeks to maintain POC where risk of infection is low, reduce disease spread and severity in high risk areas, protect uninfested watersheds, and reestablish the tree species where appropriate. Techniques such as road closures, sanitation treatments and washing vehicles are routinely used. Successful breeding of POC with various degrees of resistance to *P. lateralis* has been an encouraging recent development. The species' range has been divided into breeding zones, and seed orchards of resistant parents have been established for some. Field trials have been established on a range of cooperator's lands. For some zones, seed for reforestation and restoration is now available, and planting has begun. As plantings of genetically resistant POC reach reproductive maturity, dispersal of pollen and seed will help increase number and frequency of resistant trees in neighboring forests. A current challenge involves finding opportunities to deploy resistant stock on federal lands where planting has declined due to decreased harvests and increased dependence on natural regeneration in silvicultural prescriptions.

Restoring a fallen giant – The American chestnut
W. L. MACDONALD (1), R. B. Mann (2)
(1) West Virginia University, Morgantown, WV, U.S.A.; (2) The American Chestnut Foundation, Mount Sterling, KY, U.S.A.
Phytopathology 100:S167

The early 20th century introduction of *Cryphonectria parasitica* into eastern North America forests resulted in a host-parasite interaction that eliminated the American chestnut (*Castanea dentata*) as a forest tree and had unparalleled ecologic, economic and sociologic consequences. Fortunately, the American chestnut has been saved from extinction by its ability to sprout from surviving roots. Restoration of the American chestnut, although a daunting task, may be possible as a result of a concerted breeding program and research designed to diminish the virulence of the fungus. For over 25 years, The American Chestnut Foundation (TACF) has conducted and fostered research to employ a back-cross breeding approach to generate hybrid chestnuts that possess phenotypically-like American chestnut but carry resistance genes from their Oriental relatives. As these trees are developed, they will be released to selected sites across the original chestnut range. State TACF chapters also will contribute locally adapted germplasm for this purpose. These plantings represent small interbreeding populations that will generate resistant seed. The discovery of hypoviruses that debilitate *C. parasitica* may be instrumental to the survival of the small developing populations, especially if the trees do not possess adequate levels of resistance. Any introduction program would be shortsighted if it failed to acknowledge the constant threat posed by *C. parasitica.*

Sudden Oak Death and the future of California coastal forests
D. RIZZO (1), M. Garbelotto (2)
(1) University of California-Davis, Davis, CA, U.S.A.; (2) University of California-Berkeley, Berkeley, CA, U.S.A.
Phytopathology 100:S167

Sudden oak death (SOD) is an emerging infectious forest disease caused by the recently discovered generalist pathogen *Phytophthora ramorum*. Lethal infections are concentrated in several ecologically important species, including tanoak (*Lithocarpus densiflorus*) and various oak species (*Quercus* spp.). The disease has killed potentially millions of trees in coastal forests of California and may be changing the ecological dynamics and biodiversity of these systems. Understanding the ecology of SOD and its long-term impacts on forests requires integrating knowledge of feedback among hosts, the pathogen and the environment. Which plant species will be successfully recruited in the face of SOD and how subsequent successional patterns will develop are important questions for forest managers and conservation biologists. Development of short-term and long term management strategies for SOD in California and Oregon coastal forests is still in the early stages. Options being tested include tree removals, fire, chemical treatments, and host resistance. These management strategies must be evaluated at different spatial scales and in context with long-term management goals and policies.

Professionalism/Outreach

The APS Public Policy Board: New Challenges for Phytopathologists

Opportunities for plant pathology funding and regulatory policy priorities
K. EVERSOLE (1)
(1) Eversole Associates, Bethesda, MD, U.S.A.
Phytopathology 100:S167

Public policy affects virtually all aspects of the diverse scientific enterprise from providing education and training for careers in science to funding research projects to applying results from fundamental research to the field. Changes in program priorities by national and international funding bodies and modifications in regulatory requirements affects the everyday world of the plant pathology, sometimes making it easier for conducting science and sometimes more difficult. While agricultural research funding faces many challenges, many opportunities exist for plant pathologists to influence policy. The APS has a diverse tool kit for understanding the interrelationship between public policy and the science of plant pathology as well as for providing advice, suggestions, and assistance to improve the conditions under which scientists must operate. To gain the level of funding and breadth of participation necessary to achieve success for APS priorities, efforts are underway as well for building national and international collaborative efforts that leverage scarce research funding across multiple agencies and countries. An overview of these opportunities will be presented along with several examples of how individual scientists can become involved.

The National Plant Microbial Germplasm Collection
A. BENNETT (1)
(1) University of Arkansas, Fayetteville, AR, U.S.A.
Phytopathology 100:S167

Culture collections of plant-associated microbes represent an essential foundation for U.S. science. Microbial collections are a resource used to solve a myriad of practical challenges to our agricultural and environmental systems and play diverse and critical roles in understanding plant resistance to diseases. Collections provide a critical link between past and present disease epidemics, facilitate identification of emerging diseases, and are useful in developing strategies to control plant diseases that impact the vitality of the U.S. agricultural sector. Our microbial culture collections are at risk however because the United States lacks a coordinated national system to protect, preserve and enhance these valuable resources. The APS Public Policy Board (PPB) supports the formation of a National Plant Microbial Germplasm System (NPMGS). This emphasis comes after two workshops and a strategic plan developed by the APS ad hoc committee on culture collections. An overview of the proposed NPMGS structure, its administrative framework, and its searchable common cyber-database will be presented. Success stories resulting from proactive characterization of culture collections and "missed opportunities" will be presented by APS members. This special session is a joint effort sponsored by the APS-PPB and the APS Collections and Germplasm Committee.

Looking ahead in genomics of plant-associated microbes
S. H. HULBERT (1), J. E. Leach (2)
(1) Washington State University, Pullman, WA, U.S.A.; (2) Colorado State University, Fort Collins, CO, U.S.A.
Phytopathology 100:S167

In recent years, the APS Public Policy Board has helped funding agencies prioritize and gain support for microbial genome sequencing projects. Funding agencies will likely be devoting fewer resources to these types of projects in the future. Rapid advances in sequencing technology are making microbial genome sequencing more feasible for much less money. Individual sequence reads keep getting longer and cheaper, and other advances, like the ability to read both ends of DNA fragments, are allowing for more affordable assembly into relatively complete genome sequences. Some fungal and oomycete genome sequences can now be derived for tens, instead of hundreds, of thousands of dollars, and bacterial genome sequences can be completed even more cheaply. Genomic sequencing is now included in biology oriented, individual investigator grant proposals instead of just proposals focused on completing

the sequence. With the growing amount of microbial sequence available and other types of information associated with the sequence (e.g. polymorphism, expression data, etc.), the PPB considers processing, storage, accessibility and utilization of the data to be an important issue in the near future. The PPB is sponsoring a symposium at this meeting 'Integrated Microbial Bioinformatics' to discuss the development of integrated databases and bioinformatic support to help plant pathologists access and use this information.

Microbial-plant interactions: Human pathogens on plants
J. BARAK (1)
(1) University of Wisconsin, Madison, WI, U.S.A.
Phytopathology 100:S168

Fresh produce has become the most likely contaminated food leading to human disease, negating the paradigm that food-borne human pathogens are associated primarily with animal products. Recalls and litigation cost the produce industry millions and impact every industry sector. Uncertainties about our ability to prevent future contamination throughout the supply chain haunt U.S. producers, processors, retailers, and regulators. As a result, increasing pressure is being placed on the government to institute improved, science-based food safety standards and audit compliance programs. Fundamental and practical research is needed to identify best management practices and to determine the contamination routes, environmental survival, and interactions between human pathogens and plants. Plant pathologists are a valuable scientific resource that can drive discovery and design of effective solutions to microbial contamination of food plants. PPB has actively sought to bring the expertise of plant pathologist to the attention of FDA officials grappling with a mandate to create food safety regulations on farms. Through this effort, FDA officials attended the 2009 Annual Meeting and subsequently, hosted a listening session of APS members currently involved in food safety research in Nov 2009. A joint effort between FDA-APS/PPB has planned successive special symposium to be held at the 2010 Annual Meetings of APS and the International Association of Food Protection.

Policy making up close: Reflections of the APS - Office of Science & Technology Policy Fellow
M. PALM (1)
(1) USDA APHIS PPPQ, Molecular Diagnostic Laboratory, Beltsville, MD, U.S.A.
Phytopathology 100:S168

The Office of Science and Technology Policy (OSTP), in the Executive Office of the President, was established in 1976 to advise the President and Executive Branch on science and technology effects on domestic and international affairs, to ensure that the Executive Branch policies are informed by sound science, and to coordinate the implementation of the President's science and technology policies across agencies, states, and stakeholders. The APS Public Policy Board worked with OSTP to establish an APS sponsored OSTP fellow and Dr. John Sherwood served as the first Fellow in 2008–2009. The second Fellow at OSTP, starting in April 2010, is Dr. Mary E. Palm. Dr. Palm will discuss her activities at OSTP including initiatives of interest to APS members such as food safety, scientific collections, and education.

EPA from the inside: Report from the APS-EPA Fellow
F. P. WONG (1)
(1) University of California, Riverside, Riverside, CA, U.S.A.
Phytopathology 100:S168

There are a wide range of regulatory issues that affect plant disease management in the U.S. and working with the EPA through APS and its Public Policy Board is a mutually beneficial interaction that helps APS members and our stakeholders. APS PPB is in the process of establishing a Subject Matter Expert to serve as a resource for EPA on issues affecting plant disease management. Historically, EPA has had the most impact on plant pathology through the regulation of chemical pesticides; with new development in technology, EPA is now faced with the challenges of regulating the use of biopesticides and biocontrol agents and well as the deployment of genetically modified organisms for crop protection. Issues that APS Public Policy board has identified recently as those of interest include: new pesticide spray drift regulation, the use of fungicides for plant health promotion, the withdrawal of maneb and review of other EBDC fungicides for re-registration, the status of demethylation inhibitors (DMIs) as endocrine disruptors, fungicide resistance impacts and data requirements for plant-incorporated protectants. APS meeting symposia involving EPA scientists addressing issues such as regulatory processes that affect pesticide registration and the use of crop protection tools and fungicide resistance development are also being planned for the near future.

Prepare for Your Future: Career Opportunities After Graduate School: Part 2 - Extension

Is extension right for you?
J. L. BECKERMAN (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S168

Extension is a model of technology transfer where university-developed knowledge is delivered directly to people where they live and work; Extension plant pathology focuses on providing growers with research-based information to guide their disease management decisions. Successful implementation of a research-based extension program requires a unique skill set that allows the extension specialist to communicate with university and industry scientists, crop protection companies, and growers of varying skill levels and education backgrounds. Many of the valuable habits, traits, and skills necessary for effective extension programs are not acquired through traditional graduate education. However, all of them can be learned and can improve the likelihood of developing a successful extension program.

Extension jobs from MS to PhD: Acquiring the skills and developing the resume to get the job you want
B. J. JACOBSEN (1)
(1) Montana State University, Bozeman, MT, U.S.A.
Phytopathology 100:S168

Extension Plant Pathology has provided me a wonderful and diverse career for 37 years. During my Ms degree, I determined that an Extension/Research faculty position was to be my chosen career path. During my graduate training I took courses in communications (public speaking, print, radio and TV) and worked with faculty extension plant pathologists on development of fact sheets, recommendation guides, and extension education programs. Equally important was the development of diagnostic skills both in the field and laboratory. Experience provided by faculty mentors in appraising field situations and understanding clientele needs has been critical to a successful career. If you want a job in extension work with extension professionals, get practical experience and show you have a love for extension teaching. References from these extension professionals will be critical in getting an extension position. It is important to understand that while in graduate school you cannot anticipate what career opportunities will be available either upon graduation or during your professional career. My consul is to be well prepared by thoroughly understanding plant disease diagnosis and control and have a firm foundation regarding the biology of the different types of plant pathogens, epidemiology, pesticides and pesticide application. In addition, understanding the historical and practical basis for extension education and 4H can give you a "leg up" in obtaining that extension job.

A year in the life of a diagnostician
G. E. RUHL (1)
(1) Purdue University, West Lafayette, IN, U.S.A.
Phytopathology 100:S168

Do you enjoy mysteries and problem solving? Are you interested in plant health? Do you like to share what you have learned with others? Are you good at creative writing and multi-tasking? If so, a career as a plant disease diagnostician might just be your answer to a dream job. Today's diagnosticians utilize molecular, serological, biochemical and traditional methods to sleuth out the causal agents of plant disease on *all* manner of plant problems, on *all* commodities, for a diverse clientele. As members of the National Plant Diagnostic Network (NPDN), Land Grant University (LGU) diagnosticians are networked to assist in the detection and identification of exotic and invasive plant diseases. NPDN diagnosticians are also provided with opportunities to attend diagnostic training workshops provided by USDA/APHIS/PPQ on detection methods for regulated pathogens. Incorporating new diagnostic techniques into the clinic as well as providing first reports on new diseases diagnosed in the lab encourages intellectual as well as professional growth. Diagnosticians also often collaborate with extension specialists and state regulatory personnel for transfer of knowledge through teaching efforts and to develop first detector educational programs for numerous groups of stakeholders including Extension Educators, growers, consultants, inspectors, students, homeowners and others.

A year in the life of a county extension agent

T. C. STEBBINS (1)
(1) University of Tennessee, Chattanooga, TN, U.S.A.
Phytopathology 100:S169

A County Extension Agent has come a long way since the character Hank Kimball on the classic television show “Green Acres”. The extension agent is the direct link between the county clientele and the University. The agent organizes timely meetings and workshops to distribute new information. The agent partners with many non-profit organizations to deliver programs through existing channels. The agent trains volunteers to maximize the outreach. Master Gardener training usually occurs in the winter so volunteers are ready to help with spring and summer programs. Groups such as Habitat for Humanity, area food banks, and local nature centers use the trained extension volunteers. The volunteers also present seminars and talks that a county agent might ordinarily give. They speak to garden clubs, church and school groups. They help with community gardens and local beatification projects that are visible to the community. The agent also advises other groups like the landscapers, beekeepers and herb clubs. These groups hold regular meetings that enrich public education. They spread university extension information as well. The agent answers many phone calls and emails regarding plant problems. A Master Gardener hotline helps with this outreach as well. Hopefully an agent can also get out of the office and travel out into the county at times. This personal contact is rewarding for both parties.

Starting an extension specialist career from the ground up

L. J. DU TOIT (1)
(1) Washington State University, Mount Vernon, WA, U.S.A.
Phytopathology 100:S169

You recently completed your PhD degree in plant pathology and successfully applied for a position as an extension specialist in plant pathology at a land grant university – your dream job. However, you have not previously worked with the crops for which you’ve been assigned responsibility. How do you begin to establish a successful career as an extension specialist? How do you determine areas of research on which to focus your program? Who are your stakeholders? How do you effectively connect with (sometimes very diverse) stakeholders? How do you set up productive collaboration with the research community, county extension educators, growers, and private industry? What sources of funding are available to support your research and extension program? How do you resolve or mitigate conflicts of interest among competing stakeholders and collaborators? What steps can you take to ensure objectivity when dealing with politically sensitive issues? How can you successfully adapt your program as new (and sometimes urgent) disease problems develop? How do you seek constructive mentoring and professional development opportunities to ensure continued growth professionally and personally? This presentation will use specific examples to illustrate some of the skills, resources, attitudes, and methods that contribute to building a successful career as an extension specialist in plant pathology.

2009 Southern Division Meeting Abstracts

The following abstracts were presented at the joint meeting of the APS Southern Division and the Southern Association of Agricultural Scientists (SAAS) in Atlanta, Georgia, February 1–2, 2009. These abstracts are in addition to those published in the 2009 June Phytopathology Supplement. The abstracts are arranged alphabetically, by first author's name.

Association of fern distortion syndrome with endophytic bacteria and the use of Benlate

J. W. KLOEPPER (1), J. A. McInroy (1)
(1) Dept. Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
Phytopathology 100:S170

Fern distortion syndrome is a wide-spread problem in commercial production of Leatherleaf fern (*Rumohra adiantiformis*) in Costa Rica. Previous studies in Florida suggested that the main symptom of frond distortion was associated with history of Benlate use on this vegetatively propagated plant and stimulation of deleterious bacteria. Field and greenhouse tests were designed to confirm or refute the previous suggestion. Paired sampling of 10 ferns with distorted and 10 with normally shaped fronds was done at 6 commercial ferneries in Costa Rica. Populations of total bacteria and fluorescent pseudomonads were assessed from the rhizosphere and from inside (endophytic) rhizomes. Samples were also collected three times from two ferneries in Florida, with and without Benlate history, and the populations of bacteria in rhizosphere determined; in addition, at one time, populations of bacteria on rhizomes and inside rhizomes were determined. Results from Costa Rica revealed significantly greater populations of total bacteria inside rhizomes of ferns with distorted fronds at 5 of 6 locations, and higher populations of fluorescent pseudomonads at all locations. In Florida, significantly lower populations of fluorescent pseudomonads were found at all three sample times in rhizospheres of plants never treated with Benlate than in distorted ferns propagated from sources treated with Benlate. Also, higher populations of fluorescent pseudomonads and total bacteria were found on the surface and inside rhizomes of ferns propagated from sources treated with Benlate. Hence, our results support the previously published suggestion that distortion of fronds is associated with use of Benlate and increased populations of fluorescent pseudomonads.

Treatment of Leatherleaf fern with Benlate systemic fungicide increases populations of total and alleopathic endophytic bacteria

J. W. KLOEPPER (1), J. A. McInroy (1)
(1) Dept. Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
Phytopathology 100:S170

Using molecular techniques, we previously reported that increased populations of Pseudomonas spp. were associated with Benlate use on Leatherleaf fern (2007 Phytopathology 97: S182). The current study was done to confirm and extend the previous work, using isolation techniques. All Rhizomes of Leatherleaf fern were collected from a commercial fernery in Florida where Benlate was never used. Some rhizomes were planted and grown until 3 fronds were present on each plant, and these plants were used in a spray experiment containing 8 replicate plants of 3 treatments: Benlate WP, Benlate DF, and water. Other rhizomes were directly used in a drench experiment containing the same 3 treatments plus a 6-hr-old preparation of Benlate DF. In both experiments treatments, all applications of Benlate resulted in significantly greater populations of total bacteria and fluorescent pseudomonads in the rhizosphere, on the rhizome surface, and inside rhizomes 2–4 weeks after application. Benlate treatment also resulted in significantly more deformed root hair tips and in enhanced populations of pseudomonads inside petioles 7 weeks after treatment. The percentage of allelopathic endophytic bacteria, based on testing whole bacterial cells and cell-free metabolites on cucumber seedlings, was significantly increased by Benlate. Hence, Benlate changes the microbial community and increases virulence of the community in a perennial plant.

Applications of Benlate systemic fungicide on banana reduce plant growth and increase endophytic bacteria

J. W. KLOEPPER (1), C. Ramírez (1)
(1) Dept. Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
Phytopathology 100:S170

Benlate systemic fungicide has been linked to increased populations of endophytic bacteria in Leatherleaf fern. Banana, like Leatherleaf fern, develops rhizomes which contain endophytic microorganisms that will persist with the next crop. Two experiments were conducted to determine if Benlate applications change endophytic bacteria and alter plant growth of banana. Micro-propagated commercial banana plants were transplanted into field soil three months prior to treatment. Each experiment consisted of 8 replications, one plant each, with 6 treatments: spray with Benlate WP, Benlate DF, or water; drench with Benlate WP, Benlate DF, or water. Six months after Benlate application (experiment 1), all applications of Benlate resulted in significant reductions in height, shoot weight, and root weight of banana plants (P = 0.01). These reductions in plant growth were accompanied by changes in the populations of endophytic bacteria. Benlate treatment consistently increased populations of total bacteria and 3 of the 4 Benlate treatments increased populations of fluorescent pseudomonads inside pseudostems. Experiment 2 was destructively sampled 15 months after Benlate application. Compared to the appropriate controls, all Benlate treatments resulted in significant reductions in stem caliper, stem diameter, height, and weights of shoots, roots, and rhizomes. In addition, plants from all Benlate treatments had higher populations of endophytic fluorescent pseudomonads and total bacteria. Overall, the results indicate that Benlate increases populations of endophytic bacteria in banana, as it did on Leatherleaf fern. However, while the effect on Leatherleaf fern was distortion of frond shape, on banana, the effect is an overall stunting of plant growth and development.

Evaluation of commercially available plant growth-promoting rhizobacteria (PGPR) and plant extracts on sheath blight disease of rice caused by *Rhizoctonia solani*

K. Vijay Krishna Kumar (1), S. Krishnam Raju (2), M. S. Reddy (1), J. W. Kloepper (1), K. K. Lawrence (1)
(1) Dept of Entomology & Plant Pathology, Auburn University, Auburn, AL; (2) Dept of Plant Pathology, Andhra Pradesh Rice Research Institute, Maruteru, India
Phytopathology 100:S170

Sheath blight disease of rice caused by *Rhizoctonia solani* is a major production constraint in all rice producing areas of the world. The annual losses due to sheath blight are estimated to be 25% under optimum conditions of disease development. Disease management is currently focused on extensive use of fungicides which has created concerns about environmental pollution, pathogen resistance and escalating costs. Field trials were conducted during rainy seasons of 2005 and 2006 in randomized block design with three replications to assess the commercially available bio-pesticide products for their effect on sheath blight. Products evaluated were Achook (Azadirachtin), Biotos (Plant activator), Tricure (Azadirachtin), Ecomonas (*Pseudomonas fluorescens*) and Bavistin (Carbendazim) in 2005 and Biofer (Plant extract), Biotos, Defender (Plant extract), Ecomonas, Florezen P (*P. fluorescens*), Trichozen (*Trichoderma viride*) and Bavistin in 2006. Products were applied three times as foliar sprays after appearance of first symptoms initially and repeated at 10 days interval. The disease severity was measured by adopting Highest Relative Lesion Height (HRLH) at 90 days after transplanting. The chemical (Bavistin) reduce disease severity 52% and 50% compared to the control. Corresponding reductions in disease severity with the bio-pesticides ranged from 22% to 48% in 2005 and from 15% to 31% in 2006. Specifically with PGPR, the disease reductions ranged from 14% to 38% compared to the control in both the years. Grain yields were assessed at 120 days after transplanting and significantly increased grain yields (3,901 and 1,938 kg/ha) over control (2,690 and 1,550 kg/ha) were obtained with PGPR in 2005 and 2006 respectively. Our results showed that there is a scope for effective management of sheath blight disease with the use of the currently available PGPR and other products that are available under the conditions evaluated.

Changes in chrysanthemum rhizosphere bacteria related to steam treatment and reduced plant growth

A. Suárez (1), M. Ramírez (1), J. Pérez (1), N. Cardona (1), J. Calle (1), C. RAMÍREZ (1)

(1) Institute of Biology, Universidad de Antioquia - Centro de Innovación de la Floricultura Colombiana, Colombia

Phytopathology 100:S171

Steam treatment of soils is common in commercial production of chrysanthemum in Colombia. Although highly effective, the beneficial effect of steam shortly disappears, and reductions of plant growth and vigor occur after the first harvest. Affected plants do not exhibit classical disease symptoms, nor are pathogens isolated from them. In attempts to overcome the growth reduction, growers re-apply steam frequently, thereby increasing production costs and potentially damaging soil health. We hypothesized that reduced plant growth, following steam treatment, is associated with increases in deleterious bacteria. To test this hypothesis, populations of total culturable and aerobic endospore-forming (AEFB) bacteria and fluorescent pseudomonads were determined in rhizosphere soil during three different planting cycles after steam treatment and compared to populations in a control chrysanthemum-cultivated soil supporting satisfactory plant growth and lacking any history of steam treatment. Significantly higher populations of all three groups were recorded in the third round of planting. However, for the second round, when the plant growth (high and fresh weight) was already significantly reduced, only increases in fluorescent pseudomonads were significant. For the first round after steam treatment, when plant growth was optimum, the populations of total bacteria and AEFB were higher in treated than in non-treated soil, whereas fluorescent pseudomonads were similar. Significant negative correlations were found between plant growth and the population of each of the bacterial groups evaluated, and the correlation coefficient was greatest for fluorescent pseudomonads. Tests for potential bacterial deleterious traits have revealed a higher proportion of indole-acetic acid-producing morphotypes of fluorescent pseudomonads in treated soils, whereas similar numbers were found for HCN production.

2009 Caribbean Division Meeting Abstracts

Abstracts presented at the joint meeting of the APS Caribbean Division and The Florida Pathological Society in Orlando, Florida, May 16–19, 2009. The abstracts are arranged alphabetically, by first author's name.

Monitoring resistant populations of *Xanthomonas citri* subsp. *citri* and epiphytic bacteria on young citrus trees treated with copper or streptomycin

F. BEHLAU (2), J. B. Jones (2), J. H. Graham (1)
(1) Citrus Research and Education Center, University of Florida, Lake Alfred, FL; (2) Plant Pathology Department, University of Florida, Gainesville, FL
Phytopathology 100:S172

Since Florida's citrus canker (*Xanthomonas citri* subsp. *citri*, Xcc) eradication program was halted in 2005 attention has focused on management strategies that include the use of bactericides such as copper and streptomycin for disease control. Widespread use of these chemicals in citrus industries elsewhere in the world has led to development of resistant strains of Xcc. Cu and Sm resistance were monitored in Xcc and epiphytic bacterial populations on citrus trees repeatedly sprayed with these chemicals for control of citrus canker. Copper hydroxide (Cu, Kocide 3000) or streptomycin sulphate (Sm, Firewall) were sprayed on foliage of young 'Ray Ruby' grapefruit every 21 days from March to October 2008. Mature canker-symptomatic and non-symptomatic leaves were sampled monthly to assay for resistant Xcc and epiphytic bacteria, respectively. Leaves were washed with MGY broth + 1 mg/L of $CuSO_4$ for 2 hrs using 10 mL of liquid medium/g of leaf and plated on semi-selective medium MGY-KCC + Cu or Sm for isolation of resistant Xcc or on MGY + Cu or Sm for monitoring resistant population of epiphytic bacteria. No Cu or Sm resistant strains of Xcc were isolated. No major differences in total epiphytic bacterial population were observed among treatments over time in comparison to the check. However, Cu and Sm sprays increased the ratio of epiphytic bacterial population with resistance to these chemicals. Overall, the Sm resistant bacterial populations were proportionally lower than Cu resistant bacterial population.

Survival of *Xanthomonas citri* subsp. *citri* on symptomatic fruit under prolonged ambient and cold storage conditions

G. Bonn (1), E. TAYLOR (3), T. Riley (2), T. Gottwald (3), T. Schubert (1)
(1) Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Gainesville, FL; (2) USDA, APHIS, PPQ, Orlando, FL; (3) USDA, ARS, US Horticultural Research Laboratory, Ft. Pierce, FL
Phytopathology 100:S172

Live cells of *Xanthomonas citri* subsp. *citri* (*Xcc*) were detected by excising canker lesions from commercial fresh-packed grapefruit, macerating them in phosphate buffer followed by dilution plating onto a semi-selective agar medium (KCB). After 4–5 days incubation at 28°C, separate colonies were counted at 100X using a dissecting microscope. Confirmation of Xcc was by the use of Agdia's ImmunoStrip® for suspect plate colonies and a bioassay by needleless infiltration of leaf lamina of young 'Duncan' grapefruit seedlings. *Xcc* was detected from lesions on fruit held at both ambient and 5°C for up to 100 days in storage. There was a general trend for the percentage of *Xcc* positive lesions and actual bacterial populations to decrease with storage time. Populations of *Xcc* decreased faster at ambient temperature than at 5°C, possibly due to the higher metabolic activity of cells or microbial competition at the elevated ambient temperature. The low numbers of viable canker bacteria associated with peel lesions in grapefruit, especially over time, suggest that the risk of canker transmission from them is extremely low in comparison to active lesions on the leaves, stems and fruit during the growing season.

Distribution of *Candidatus* Liberibacter asiaticus in huanglongbing infected citrus trees

R. H. BRLANSKY (1), K. S. Pelz-Stelinski (1)
(1) University of Florida, CREC
Phytopathology 100:S172

The distribution of the huanglongbing (HLB) associated *Candidatus* Liberibacter asiaticus (Las) bacterium in mature field infected citrus trees was evaluated. The number of tissue samples collected per tree ranged from 16–32 and included: fruit peduncles, bark phloem, and symptomatic leaf midribs and petioles taken from throughout the tree canopy. Overall, the percentage of Las-positive plant tissue samples obtained ranged from 23–44% based on real-time PCR results. Although samples taken from bark phloem varied, phloem from one-year old bark consistently tested positive for Las. Similar variation in the detection of Las occurred in samples obtained from leaf petioles and fruit peduncles. The percentage of positive leaf petioles ranged from 0–100%, while fruit peduncles ranged from 17–56%. Additional replicates continue to be collected in order to firmly establish whether one-year old bark phloem is consistently positive when another part of the tree has tested positive for Las via PCR. These results indicate that distribution of the HLB-associated pathogen varies widely within symptomatic, PCR-positive citrus trees and thus illustrate the importance of obtaining multiple samples from trees where an infection is suspected.

Infectious clones and characterization of a previously unreported bean-infecting begomovirus from *Rynchosia minima* (L.), an endemic legume species from Puerto Rico

J. K. BROWN (2), M. Rehman (1), A. M. Idris (2)
(1) Department of Microbiology, Hazara University, Mansehra, Pakista; (2) Department of Plant Sciences, The University of Arizona, Tucson, AZ, USA
Phytopathology 100:S172

R. minima plants exhibiting mild mosaic symptoms that were reminiscent of begomovirus infection were observed in Puerto Rico during the summer, 1997. Total nucleic acids were extracted from symptomatic *R. minima* leaves using the CTAB method. The viral single-stranded DNA was subjected to rolling circle amplification. The *Sac*I-linearized, multimeric DNA band was ligated into *Sac*I-digested pGEM7Zf+ and cloned. Eight clones bearing a ~2.6 kbp fragment were sequenced using primer walking. Analysis of the resultant sequences indicated that five and three of the clones were DNA-A and DNA-B components, respectively. The genome organization and number of open reading frames (six) was typical of other bipartite begomoviral genomes from the Western Hemisphere. Comparative analysis of the DNA-A (n = 5) and DNA-B (n = 3) component sequences shared 98–99% and 99% nucleotide (nt) identity, respectively. Inspection of the common region (CR) revealed that they were cognate components, and shared an identical iteron. The DNA-A component shared 80% nt identity with its closest relatives, *Macroptilium mosaic Puerto Rico* virus and *Rhynchosia golden mosaic virus*. The DNA-B component shared 64% and 62% nt identity with RhGMV and *Cabbage leaf curl virus*, respectively. This previously undescribed begomovirus species is herein named Rhynchosia mild mosaic virus (RhMMV). Clones of the RhGMV DNA-A and DNA-B component were released with SacI and biolistically inoculated to *R. minima* and bean (*Phaselous vulgaris*) seedlings.

R. minima seedlings developed mild mosaic symptoms like those observed in field-infected bean plants, thereby fulfilling Koch's postulates, and the bean seedlings developed severe green-yellow mosaic symptoms, confirming that the virus also infects bean. This previously undescribed virus could pose a serious threat to bean crops in the Caribbean region.

Developing an effective international education program for management of *Ralstonia solanacearum* Race 3 biovar 2

P. G. CHAMPOISEAU (1), J. B. Jones (1), C. Allen (3), T. M. Momol (2)
(1) University of Florida, Department of Plant Pathology, Gainesville, FL, USA; (2) University of Florida, District Directors Office, Gainesville, FL, USA; (3) University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI, USA
Phytopathology 100:S173

Because it threatens both potato and ornamental production, *Ralstonia solanacearum* Race 3 biovar 2 (R3bv2) is considered a serious quarantine pest in Canada and Europe and is listed as a Select Agent plant pathogen in the United States, where it is subject to the strictest biosecurity regulations. Although this pathogen is not known to be established in the US, import of infected geranium cuttings from off-shore production sites has already proved to be a possible pathway for introduction. Previous accidental introductions resulted in multi-million dollar losses due to quarantine responses. Therefore it is critical to prevent further re-introduction and possible spread of R3bv2 in the US. This involves exclusion, early detection, and unambiguous identification of the pathogen at both national and international levels. This can be achieved by use of reliable diagnostic tools for the pathogen and effective phytosanitary measures; however, this is not enough. It is also essential to ensure preparedness and effective training of official regulators, diagnosticians and other individuals responsible for first detection and response to a possible R3bv2 discovery in the US. We have therefore developed an integrated education and outreach program, as part of a USDA-founded project for a better management of R3bv2. This program involves development of educational and training content by a team of experts, delivery of educational materials to target audiences by diverse means including current web-based technologies, as well as use of various evaluation tools to assess program effectiveness. Monitored access of our *Ralstonia*/bacterial wilt-dedicated website shows that stakeholders from diverse organizations both within and outside the US regularly use this resource to obtain updated and accurate information on *R. solanacearum* R3bv2 and bacterial wilt disease management.

Nitrocellulose membranes as a solid matrix for *Cucumber mosaic virus* immuno-detection and subgroup identification by RT-PCR

P. S. CHANG (1), S. A. Tolin (1)
(1) Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
Phytopathology 100:S173

Cucumber mosaic virus (CMV) is an important and widespread plant virus. The strains and isolates of CMV are highly diverse and assigned to either subgroup 1A, 1B or 2. Here we report the application of tissue blot immunoassay (TBIA) followed by reverse transcription-polymerase chain reaction (RT-PCR) for the immuno-detection and subgroup identification of CMV from various hosts and locations. Freshly torn leaves were blotted onto nitrocellulose membranes (NCM), which were used as sources of viral RNA after processing by TBIA. CMV positive samples show a purple precipitate at the blot site. A 3 mm disc was removed from the positive sites and cleaned by rinsing with Triton X-100, followed by TE buffer, then dried and added directly to RT reactions that included a reverse primer to the CMV coat protein (CP) gene. The resulting cDNA was added to PCR reactions containing forward and reverse primers for the CMV CP gene. Agarose gel electrophoresis revealed amplicons of the expected size. The subgrouping of CMV samples was predicted from sequences of PCR products and confirmed by monoclonal antibodies specific to CMV subgroups 1 and 2. Successful amplification was possible from NCM blotted and TBIA processed up to 15 months previously, but amplification levels from older blots were lower. The PCR protocol was adjusted by increasing the number of cycles for consistent results from blots older than 8 months. This method eliminates the need for leaf tissue storage or costly RNA extraction when sampling for CMV diversity or monitoring virus prevalence and incidence. NCM are thus a suitable matrix for obtaining viral RNA for RT-PCR and archival storage of viral nucleic acids, similar to Whatman's FTA® Plant Cards.

Sugarcane orange rust, an emerging disease in the western hemisphere

J. C. COMSTOCK (1), N. C. Glynn (1), L. A. Castlebury (2)
(1) USDA-ARS Sugarcane Field Station, Canal Point, FL; (2) Yystematic Mycology and Microbiology Laboratory, ARS, USDA, Beltsville, MD
Phytopathology 100:S173

Symptoms consistent with sugarcane orange rust were first observed in Florida in June 2007, these were subsequently confirmed morphologically and molecularly as being caused by *Puccinia kuehnii*, the causal agent of orange rust. This was the first documented occurrence of sugarcane orange rust in the Western Hemisphere. Since then it has been reported in Guatemala, Costa Rica and Nicaragua and has been confirmed in several other Central American and Caribbean Countries. A comparison of brown rust and its causal agent, *P. melanocephala* and *P. kuehnii*, will be presented. Orange rust has impacted both the commercial production and the cultivar development program in Florida. One major difference in the epidemiology of the two pathogens is that *P. kuehnii* tolerates warmer temperatures and orange rust severity continues throughout the summer and early fall lasting much longer than brown rust. This is significant as it means that commercial cultivars susceptible to both pathogens are impacted by either one or both pathogens depending on the month of the growing season. A cultivar that occupies 25% of the acreage in Florida, CP 80-1743, is susceptible to the disease and has had reduced cane yields. It is being withdrawn from production. Results from a comprehensive approach towards developing sugarcane cultivars resistant to orange rust that is being adopted in the Canal Point breeding program will be presented. This involves identifying and discarding susceptible sugarcane clones as early in the breeding program as possible, the development of novel screening methods and the identification of sources of resistance for breeding.

'*Candidatus* Liberibacter solanacearum' on tomato and potential losses in field production

R. D. FRENCH-MONAR (3), R. W. Wallace (2), J. A. Abad (1), T. A. Wheeler (4)
(1) APHIS-PPQ-PGQP, Beltsville, MD, USA; (2) Dept. of Horticultural Sciences, AgriLife Extension-Texas A&M System, Lubbock, TX, USA; (3) Dept. of Plant Pathology and Microbiology, AgriLife Extension-Texas A&M System, Amarillo, TX, USA; (4) Dept. of Plant Pathology and Microbiology, AgriLife Research-Texas A&M System, Lubbock, TX, USA
Phytopathology 100:S173

In August 2008, tomato (*Solanum lycopersicum*) plots in Lubbock County, TX that were utilized for a chemical test aimed at management of root knot nematode became infected with '*Candidatus* Liberibacter solanacearum'. Overall symptoms on tomato cv. Spitfire included leaf yellowing, lateral stem dieback, upward leaf curling, enlargement of stems, adventitious roots, and swollen nodes. PCR amplification was done using 16S rDNA OA2 and OI2c primers for '*Ca*. L. solanacearum' used for potato, tomato, and other solanaceous crops in New Zealand, which amplifies a 1.1 kb fragment of the 16S rRNA gene of this new species. A 1.1 kb fragment was obtained, sequenced, and found to be 99.9% identical in sequence to a '*Ca*. L. solanacearum' obtained last year from a potato production field in Texas. In the tomato field, a total of 32 plots (one-row wide, 7.7 m long) comprised of 24 plants per plot were evaluated for disease symptoms and galling by root-knot nematode. Foliar disease incidence in plots ranged from one (4.2%) to 19 (79.2%) plants showing symptoms by the last harvest date. Regression analysis was used to determine losses in yield associated with the bacterium and with root-knot nematode. Percent galling by root-knot nematode only explained 14% of the variability in yield, and 100% galling was predicted to cause a 1.5% loss in yield, based on the regression model. In contrast, for each 1% incidence in plants with disease symptoms, there was a 0.9% loss in yield. In essence, there was no yield contribution if a plant developed symptoms (R^2 = 0.41). The potential exists for '*Ca*. L. solanacearum' to be a detriment in tomato production and a source for survival of this bacterium that has been found to be associated with the Zebra Chip disease in potato, tomato, pepper (*Capsicum annuum*), and other solanaceous crops.

Diverse tomato cropping systems affect arbuscular mycorrhizal fungal community diversity and structure

E. G. JOHNSON (3), D. O. Chellemi (2), T. Wu (1), J. H. Graham (3)
(1) Division of Cell Biology, Microbiology, and Molecular Biology, University of South Florida, Tampa, FL, USA; (2) USDA-ARS, US Horticulture Research Laboratory, Fort Pierce, FL, USA; (3) University of Florida, Citrus Research and Education Center, Lake Alfred, FL, USA
Phytopathology 100:S173

In conventional agricultural systems, AMF are greatly affected by factors including soil disruption, intermittent lack of host root tissue, and plant inhibition of AMF colonization due to high soil fertilization. To determine the effect of diverse agricultural land management and crop production practices on the AMF community structure and diversity, five tomato crop production systems consisting of bahiagrass pasture cover, conventional, continuous removal of vegetation (disk fallow), organic, and undisturbed (weed fallow) were initiated. The plots were adjusted to the new management regime, except for conventional, for three or four years followed by one or two years of tomato cropping. Soil DNA samples were taken in the off season, at planting, and after harvest. Phylogenetic analysis of AMF 18S rDNA sequence combined with multivariate statistical analysis using PRIMER-E was used to compare community structure and diversity. Initial analysis shows that

bahiagrass, weed fallow, and organic land management practices support different, diverse AMF communities, while disk fallow and conventional practices greatly reduced detection of AMF sequences. Tomato cropping caused the emergence of common sequences for the *Glomus mosseae* group, in all cropping systems. Bahiagrass and weed fallow diversity were unaffected by the emergence of the *G. mosseae* group, while organic, conventional and disk fallow all converge on a low diversity community dominated by the *G. mosseae* group. Current analyses will determine if the shift in AMF community caused by tomato cropping in organic and bahiagrass plots is seasonal or persistent, and if other factors such as soil fertility and disease incidence correlate to these community changes.

Identification and characterization of powdery mildew caused by *Golovinomyces cichoracearum* on sunn hemp

S. A. JORDAN (1), G. S. Maia (1), A. J. Gevens (1)
(1) University of Florida, Gainesville, FL, USA
Phytopathology 100:S174

Crotalaria juncea, or sunn hemp, is a warm season legume grown in FL as a cover crop. In 2008, powdery mildew was observed on sunn hemp in a research field in Hastings, FL. This disease is important because it has the potential to impact the quality of sunn hemp and this powdery mildew can infect cucurbits which are grown in north FL in late summer. Fungal growth appeared first on lower, more mature leaves as white, powdery mildew colonies initially seen on upper leaf surfaces and later moving to undersides; petioles and floral parts were disease-free. As disease progressed, colonies enlarged, coalesced, and covered entire leaf surfaces; heavily infected leaves senesced and abscised. Mycelia produced white accumulations of conidiophores and conidia. Hyphae were superficial with papillate appressoria and produced conidiopohores with cylindrical foot cells that measured 48.5 × 10.0 µm. Conidia were hyaline, short-cylindrical-ovoid, lacked fibrosin bodies, borne in short chains, had sinuate edge lines with other immature conidia, and measured 22.5-40.0 × 12.5-20.0 µm. The teleomorph was not observed. The nuclear rDNA internal transcribed spacer (ITS) regions were amplified by PCR and sequenced. On the basis of morphological characteristics of the asexual state and ITS sequence data, the pathogen was identified as *G. cichoracearum*. Pathogenicity was confirmed on healthy plants. This is the first report of *G. cichoracearum* causing powdery mildew on *C. juncea.*

***Phytophthora cactorum* a serious problem on prefinished Cattleya orchid liners from Thailand**

R. T. MCMILLAN (1), A. J. Palmateer (2), R. A. Cating (2)
(1) Kerrys, Homestead, FL; (2) University of Florida, Homestead, FL
Phytopathology 100:S174

The major pathogen on Cattleya orchids in Florida and in the New and Old World countries is *Phytophthora cactorum* (Lebert & Cohn) J. Schroet., which causes Black Rot during the wet months of the year. All species of Cattleya and their interspecific and intergeneric hybrids are susceptible. *Phytophthora cactorum* infects leaves, pseudobulbs, rhizomes, and flower buds. Shipments of prefinished Cattleya orchid liners from Thailand during the monsoon season often infected with *P. cactorum*. Orchid plants with visual symptoms of *P. cactorum* were removed from the shipment and drenched with fungicides such as Banrot, Natriphene, Shield Brite, Truban, and Phyton 27, Heritage, Shield Brite, Stature, Truban, Pentathlon, Aliette, Subdue Maxx, and Insignia, in an effort to salvage some of the plants. In spite of the effort to save the orchids, the level of Phytophthora over rode the attempt to control the fungus resulting in destroying the shipments. Cattleya liner shipments during the dry season are found to be *P. cactorum* free.

Controlling angular leaf spot in Florida annual strawberry

J. C. MERTELY (1), N. A. Peres (1)
(1) University of Florida-GCREC, Wimauma, FL, USA
Phytopathology 100:S174

Angular leaf spot (ALS) is a bacterial disease caused by *Xanthomonas fragariae* that produces unsightly lesions on strawberry leaves and sepals. Leaves with numerous spots and/or vein-following lesions become blighted and die prematurely. During the 2008–2009 growing season, an epidemic of ALS occurred in the principal Florida strawberry production area west of Tampa. During the season, a replicated trial evaluating products for ALS control was conducted at the Gulf Coast Research and Education Center in Wimauma, FL. Treatments included the plant defense activator acibenzolar-s-methyl (Actigard); copper fungicides Badge, Cuprofix, IRF070, Kentan, Kocide, and Quint; hydrogen peroxide (Oxidate); and *Streptomyces lydicus* (Actinovate) applied weekly throughout the season to the foliage with a CO_2 back pack sprayer. A late-season evaluation of foliar symptoms showed that Badge, IRF070, Kentan, Kocide, and Actigard significantly reduced the proportion of leaves killed and partially blighted by *X. fragariae*. Alternating applications of Actinovate and Cuprofix also showed this effect. However, only Badge and the low rate of Actigard significantly increased marketable yield during a season with markedly high disease pressure. Future experiments may target applications to periods favorable to infection and disease spread, such as rain events associated with approaching cold fronts, and periods of prolonged overhead irrigation for freeze protection.

Cultivar susceptibility, temperature and leaf wetness durations required for lesion production by *Alternaria alternata* on tangerine and tangerine hybrids

S. N. Mondal (1), L. Timmer (1), M. M. DEWDNEY (1)
(1) University of Florida
Phytopathology 100:S174

Alternaria brown spot, caused by *A. alternata*, is an important disease of tangerines and their hybrids in many citrus-producing regions. A prediction model for fungicide applications, the Alter-Rater, was developed previously, but it was unknown whether the relationship between temperature and leaf wetness duration (LWD) would be consistent across all tangerine hybrids. We tested the LWD and temperature relationships on 5 tangerine and tangerine hybrids: Dancy, Minneola, Murcott, Nova and Sunburst. The LWDs were 2, 4, 8, 16, 24 and 30h at temperatures of 20, 24, 28 and 32°C. The rating scale for the number of lesions/leaf was: 0 = 0; 1 = 1-2; 2 = 3-5; 3 = 6-10; 4 = 11-15; 5 => 15 lesions/leaf and the data were taken from 15 leaves. The experiment was an incomplete block design. Cultivar differences were observed; lesions formed on Minneola and Dancy with as little as 2h of leaf wetness at all temperatures. Lesions were observed on Murcott, Nova and Sunburst with 4h of leaf wetness. The optimal temperature range for lesion production was 24 and 28°C for all LWDs. On the more susceptible Minneola and Dancy, 24h LWD was required to reach the max. lesion rating, but that level was never reached on Murcott, Nova and Sunburst even with 30h of leaf wetness. The results should be incorporated into the Alter-Rater model so that unnecessary sprays are not applied to less susceptible tangerine and tangerine hybrids.

Identification of the Florida torreya canker pathogen

L. L. MOUNT (1), J. A. Smith (1)
(1) University of Florida
Phytopathology 100:S174

The Florida torreya (*Torreya taxifolia* Arn.) is a conifer of the Taxaceae. The native population is restricted to within 20 miles of the Apalachicola River in northern Florida and southern Georgia. Torreya wood is resistant to decomposition. For this reason, it was lumbered for railroad ties in the 19th century. Surveys conducted prior to 1970 detail a dramatic reduction of population numbers, size and health. Historically, trees reached 18 m at maturity. Of the 58 trees surveyed in November 2008, no individual surpassed a height of one meter. Disease symptoms associated with this decline are leaf spot, shoot tip dieback, and cankers. Fungal cultures were isolated from symptomatic tissue and from the initial 58 trees tested, cultures of *Fusarium* spp. and *Botryosphaeria* spp. were each isolated 20 times. The same tree sample often produced both genera. Products were amplified by polymerase chain reaction using internal transcribed spacer region rDNA (ITS-rDNA) specific primers and the sequences obtained were compared to those deposited in the GenBank database. Four unique sequences of *Fusarium* spp. and *Botryosphaeria obtusa* were identified. *Fusarium solani* and *F. lateritium* matched GenBank sequences to the species level. *Fusarium lateritium* was previously identified as the causal agent of the leaf spot, but was cultured during this study directly from cankered tissue. Two species of *Phomopsis*, as well as *Diaporthe, Lasiodiplodia*, and *Hypoxylon* were also infrequently isolated and identified in the same manner from dying shoots and cankers sampled in November 2008 and January 2009. Inoculations of as many of these genera as possible were conducted on torreyas grown using sterile tissue culture at Atlanta Botanical Gardens in April 2009.

The impact of silicon soil amendments on cucumber anthracnose in the greenhouse

J. PALENCHAR (1), S. Taber (1), L. E. Datnoff (2), and A. J. Gevens (1)
(1) Department of Plant Pathology, University of Florida, Institute of Food and Agricultural Sciences, Gainesville, FL; (2) Current address: Dept. of Plant Pathology and Crop Physiology, Louisiana State University
Phytopathology 100:S174

Cucumber is an economically important crop in Florida and in other parts of the U.S. In Florida, one of the most common diseases on cucumber is anthracnose, caused by the ascomycetous fungus *Colletotrichum orbiculare*. Anthracnose can cause serious yield and quality losses, and produces symptoms on all aboveground plant parts at any stage of growth. Organic producers have limited options for anthracnose control. The use of silicon (Si) as a tool for disease control has been established in other crop systems. In the greenhouse, the control of anthracnose was demonstrated on 2-week-old 'Straight Eight' cucumber seedlings by amending soil (organic Fafard FOF

30) with a high rate of Si (Vansil W50). Treatments included cucumber seeds planted into 1) Si-amended soil (600 kg Si/ha) + no *C. orbiculare* inoculation 2) Si-amended soil (600 kg Si/ha) with *C. orbiculare* inoculation, 3) non-amended soil with no *C. orbiculare* inoculation, and 4) non-amended soil with *C. orbiculare* inoculation. Each treatment included 5 replications and the experiment was repeated 4 times. Disease evaluations (Horsfall-Barrett scale) were recorded for leaves at 3, 7, and 14 days post inoculation (dpi) and Si levels in plant tissues were determined. Significant differences between treatments were observed at 7 and 14 dpi. Si treatment reduced disease severity on leaves by 20–60% when compared to the inoculated control. This is the first study demonstrating the efficacy of soil-applied Si for the control of cucumber anthracnose.

Diagnostics and emerging plant pathogens

M. E. PALM-HERNANDEZ (1)
(1) USDA/APHIS/PPQ, Molecular Diagnostic Laboratory, Beltsville, MD, USA
Phytopathology 100:S175

Accurate identification of plant pathogens is essential for making management decisions and determining appropriate regulatory actions. An accurate identification is facilitated when a group is well understood taxonomically based on robust systematics studies. Such studies provide information on which morphological and molecular characters are taxonomically useful and tools can then be developed to use in detection and identification. Identification of emerging plant pathogens poses a particular challenge in that they are often understudied and poorly characterized. *Phytophthora ramorum*, the causal agent of sudden oak death and ramorum blight, exemplifies how regulatory diagnostics evolve as scientific knowledge about the disease and its causal pathogen is gathered and evaluated. The discovery of new and related taxa, including *P. foliorum* that cross-reacted in the *P. ramorum* nested assay, led to the development of additional assays. The use of new markers to compare large numbers of *P. ramorum* isolates has provided a clearer picture of the genetic diversity in the pathogen and a means of tracing origins of newly found isolates. This case study is one of many examples of the importance of a strong understanding of the systematics of a group as the basis for accurate identification and the development of diagnostic tools.

Basil downy mildew in Florida: A disease of new importance

R. N. RAID (1), P. D. Roberts (4), P. F. Harmon (2), A. J. Palmateer (3), S. A. Jordan (2)
(1) University of Florida, Belle Glade, FL, USA; (2) University of Florida, Gainesville, FL, USA; (3) University of Florida, Homestead, FL, USA; (4) University of Florida, Immokalee, FL, USA
Phytopathology 100:S175

Sweet basil (*Ocimum basilicum* L.) is one of the most important herbs currently grown in Florida, with both commercial field and greenhouse production. In addition, it is one of the most commonly propagated herbs in home gardens. Fortunately, it has had very few foliar disease problems and has, for that reason, required little or no disease management. During fall 2007, a new disease was first reported on field-grown basil in south Florida. Symptoms initially appeared as a yellowing of the lower canopy, with chlorotic areas frequently delineated by leaf veins. Gray, fuzzy fungal growth was apparent on the abaxial leaf surface. The disease was subsequently reported to be incited by a species of *Peronospora*. Yield losses during this initial outbreak were near total, since preventative control measures were formerly unnecessary, and therefore, non-existent. Since the initial outbreak, basil downy mildew has become firmly established in Florida. It has been observed from all regions within the state, as well as in numerous other states. Although there is ample evidence that the disease may have been introduced on infested seed, alternative sources (i.e. from closely related hosts) have not been totally ruled out. With widely-scattered, year-round greenhouse and/or field production providing a host continuum, it is very likely that basil downy mildew will be a disease to contend with on a permanent basis. Nearly all basil varieties or types appear susceptible at this time. Management programs are currently under development. Aside from cultural practices to limit leaf wetness and hence fungal infection, preventive foliar applications of phosphonates and strobilurin fungicides have proven useful. Used together in a program, these have provided economic but not total control.

Orange rust of sugarcane: Prospects for fungicidal control

R. N. RAID (2), J. C. Comstock (1), N. C. Glynn (1)
(1) USDA Sugar Cane Field Station, Canal Point, FL, USA; (2) University of Florida, Belle Glade, FL, USA
Phytopathology 100:S175

Orange rust of sugarcane, incited by *Puccinia kuehnii*, was first observed in Florida during June 2007 on one of the industry's most important commercial cultivars, CP80-1743. This was the first report of this disease in the Western Hemisphere. It has since been reported in several other Central American and Caribbean Countries. With host-plant resistance being a worthy long-term goal, studies were initiated to investigate the feasibility of fungicides serving as an interim or supplementary management strategy. Thirteen different fungicide treatments were examined for their efficacy in controlling orange rust during the 2008/2009 growing season. Experimental units consisted of two rows of cane 15m in length replicated four times in a randomized complete block design. Fungicide treatments consisted of select candidates from two major classes of fungicides, the strobilurins (FRAC group 11) and triazoles (FRAC group 3), alone, and in combination or alternation. Fungicide applications were made using a CO_2 backpack sprayer and were initiated following canopy closure (approx. 1.5-m ht) at 21 day intervals. Rust severity in the trial area was moderately severe, with severities in excess of 30% on the distal third of the fourth leaf beneath the top-visible-dewlap leaf in the untreated check. Results indicate that the strobilurin fungicides provided the highest level of control, followed by strobilurin/triazole combinations, and finally, the triazole fungicides alone. In separate trials using the strobilurin fungicide pyraclostrobin, fungicide treatments were demonstrated capable of reducing orange rust to levels sufficient to significantly reduce yield losses by as much as 40%. While economic factors will ultimately be an important consideration, levels of orange rust control obtained in these studies show promise regarding prospects for fungicides as a potential management tool.

Fungicidal control of basil downy mildew

R. N. RAID (1)
(1) University of Florida, Belle Glade, FL, USA
Phytopathology 100:S175

Sweet basil (*Ocimum basilicum* L.) is one of the most common herbs grown by home gardeners in Florida. Commercially, basil ranks as Florida's most important potted herb and the state ranks second nationally in field production, shipping to the entire eastern seaboard. Since 2007, basil downy mildew, incited by a species of *Peronospora*, has caused considerable losses for commercial basil growers in the U.S. In the absence of control, total crop failure is common. Two fungicide field trials were conducted to determine the efficacy of various foliar applications for the control of this disease. The tests included both registered and non-registered compounds. Experimental units consisted of four rows 2m in length separated on the ends by alleyways and replicated four times in a randomized complete block design. All experimental compounds were topically applied on approximately a weekly basis using a CO_2 backpack sprayer equipped with 3 flat-fan nozzles mounted on a hand-held boom. Treatment commenced at the 4-6 leaf stage, with mildew present in the area at time of initial application. Disease severity was considered extreme. Products tested in these trials included: acibenzolar, azoxystrobin, *Bacillus subtilis*, chlorothalonil, copper hydroxide, cyazofamid, cymoxanil/famoxadone, dimethomorph, fenamidone, mandipropamid, mefenoxam, potassium phosphanates, propamocarb, pyraclostrobin/boscalid, and *Streptomyces lydicus*. All products provided for significant suppression of downy mildew early in the trials, but only a few provided for significant control by the end of the tests. Dimethomorph, mandipropamid, cyazofamid, fenamidone, and mefenoxam provided the highest levels of control, but are not currently labeled for use on basil. Of currently registered products, only azoxystrobin and the potassium phosphonates provided control levels that could be considered acceptable from a marketing perspective. No compounds licensed for use in organic herb production provided acceptable levels of mildew control when sprayed on a weekly basis in these trials.

Current distribution of Texas *Phoenix* palm decline in Florida

D. A. RESTOM GASKILL (2), N. A. Harrison (3), M. L. Elliot (3), T. R. Smith (1)
(1) Florida Dept. of Agriculture and Consumer Services, Gainesville, FL; (2) USDA-APHIS-PPQ, Sarasota, FL; (3) University of Florida IFAS, Fort Lauderdale, FL
Phytopathology 100:S175

Texas *Phoenix* palm decline (TPPD) is a fatal disease of date (*Phoenix dactylifera*, *P. sylvestris*, *P. canariensis*, *P. reclinata*), queen (*Syagrus romanzoffiana*) and cabbage (*Sabal palmetto*) palms caused by a '*Candidatus* Phytoplasma palmae'-related strain belonging to subgroup 16SrIV-D. In addition to the economic costs of disease management in nurseries and landscapes, the potential ecological impact due to reduction in *S. palmetto* populations is incalculable. TPPD was first reported and characterized in 2002 from samples obtained in Corpus Christi, Texas, and was detected in the Tampa Bay area of Florida in 2006. Surveys were conducted by the Cooperative Agricultural Pest Survey (CAPS) in 2008 and 2009 to determine the current distribution in Florida. Palms displaying characteristic symptoms of TPPD were sampled and analyzed by polymerase chain reaction assay. Phytoplasma positive samples from new locations were sequenced. Samples were also submitted by personnel from the University of Florida, Institute of Food and Agricultural Sciences (UF-IFAS) and from private landscape companies. TPPD was determined to occur at a high incidence in a three

county area (Hillsborough, Manatee, Sarasota), while it was found to be of limited distribution in seven additional counties (Pinellas, Polk, Hardee, Desoto, Highlands, Lake, Duval). Spread of TPPD is likely occurring locally through an unknown insect vector and over long distances through the transportation of infected palms.

Biosafety regulation and biotechnology: How it affects public research in Latin America and the Caribbean

M. M. ROCA (1)
(1) Zamorano University, Tegucigalpa, Honduras
Phytopathology 100:S176

While poverty in developing countries is usually linked with low agricultural output, pest and plant diseases are major factors that contribute significantly to this low productivity. Genetic engineering and transgenic's have great potential to improve crop production. However, the application and use of this biotechnology has not materialized for the public sector because of the politics associated with the regulatory process. As a result, the time, effort and expense required for commercialization of transgenic crops are way beyond what public-sector investigators can muster leaving only the private sector to accomplish this task. Restrictive regulations were established when the commercial use of transgenic crops was just beginning, and have not taken into account the more than 12 years of extensive experience gained on crops tested on more than 100 million hectares in 23 countries. This information has scientifically demonstrated that crops obtained through biotechnologies do not have risk profiles that are any different from those developed through more traditional plant breeding methods. The potential health and environmental risks originally foreseen have not materialized. Furthermore, it has been demonstrated that this biotechnology provides environmental and economic benefits. As it now stands, the current biosafety regulatory standards in place continue to delay the development and use of transgenic technology. The time has come where there is a great need to consider both the benefits and the risks of this technology, and analyze them relative to those of the present agricultural production systems especially in Latin America and the Caribbean.

Low molecular variability of Potato yellow vein virus (PYVV) isolates of *Solanum phureja* and *Solanum tuberosum* from Colombia

P. Rodriguez-Burgos (2), G. Chaves (2), L. Franco-Lara (1), M. GUZMAN-BARNEY (2)
(1) Militar University Nueva Granada, Bogotá-Colombia; (2) National University of Colombia, Bogotá-Colombia
Phytopathology 100:S176

PYVV *Closteroviridae*-Crinivirus, is a quarentine, phloem-limited potato virus, with a tripartite ssRNA(+). It causes yellowing of foliage with reduction of yield. It is found in Colombia, Peru, Venezuela and Ecuador and is transmitted by white fly *Trialeurodes vaporariorum* and tubers. To study variability, CPg of 75 isolates of PYVV from *Solanum phureja* and 50 from *S. tuberosum*, from 5 Colombian regions, was amplified by RT-PCR. Amplicons were analyzed by SSCP and 32 were sequenced directly. Ten SSCP patterns were observed (P1 to P10); P1 represented 78% of the isolates, P9 9.6% and P6 4.8%. Phylogenetic analysis of 70% of CPg produced two groups: Group I (29 isolates) and Group II (3 isolates). In group I, isolates 1084 of *S. phureja* and 1114 of *S. tuberosum*, showed evidence of possible recombination within CPg. No direct correspondence between the number of SSCP patterns and the sequence clusters was found, but P1 was present in Group 1 and P6 was found in all 3 isolates of Group II. The aa relationship dN/dS = 0.214 indicated negative selection, suggesting that PYVV has a tendency for low mutation fixation. This might be related to a selection pressure coming from the insect vector. According to variability of the CPg there are at least 3 virus variants circulating in the Country, although variability among them is low.

Asparagus as host of *Phythophtora* species prevalent in Michigan and its importance as rotational crop

L. RODRIGUEZ-SALAMANCA (1), M. Hausbeck (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 100:S176

Michigan ranks third in U.S. asparagus production, after Washington and California, with 11.200 acres that produced 12 million kg of asparagus spears in 2007. *Phytophthora* spear and crown rot has been recently identified in Michigan fields as a major limiting disease of asparagus. Although different species of *Phytophthora* have been reported in other production areas as causing disease in asparagus, only one species has been found in Michigan and has identified as *P. asparagi*. *Phytophthora* sp. isolated from vegetables and ornamentals in Michigan were tested for their ability to infect asparagus spears and cause lesions. A series of growth chambers studies were conducted to determine; (*i*) an optimum inoculation point for detached asparagus spears using three *P. asparagi* isolates when incubated at 15, 20 and 25°C, (*ii*) the ability of different *Phythophthora* sp. present in Michigan agriculture to infect asparagus spears. All the studies were conducted three times. When detached spears were wounded and inoculated at 2, 9 or 16 cm from asparagus tip and incubated at 20°C, all the inoculation points developed similar-sized lesions. However, when inoculated spears were incubated at either 15 or 25°C, the size of the resulting lesion differed significantly among the inoculation points. Among the select *Phythophthora* sp. used to inoculate asparagus spears, *P. capsici* was able to cause small lesions. Further studies that investigate pathogenicity of commonly encountered *Phythophthora* sp. is important in determining appropriate crop rotation strategies.

Fungal diversity associated with rambutan (*Nephelium lappaceum* L.) in Puerto Rico

L. M. SERRATO (2), L. I. Rivera (2), R. J. Goenaga (1)
(1) USDA TARS, Mayaguez, Puerto Rico; (2) University of Puerto Rico-Mayaguez Campus, Mayaguez, Puerto Rico
Phytopathology 100:S176

Rambutan (*Nephelium lappaceum* L.) is an exotic tropical fruit of increasing importance in international markets, and that has awakened great interest from farmers in Puerto Rico. During 2008 and 2009, fruit rot and lesions on leaves, branches, and flowers were observed in rambutan orchards through the island. To examine fungal diversity associated with rambutan, samples from different organs were collected in symptomatic and asymptomatic trees. Plant tissue was superficially disinfected and transferred to acidified potato dextrose agar to promote the development of fungi. A total of 311 fungal isolates were obtained, which include 19 genera. Based on morphology, the following species have been identified: *Beltrania rhombica*, *Botryodiplodia theobromae*, *Botryosphaeria* spp., *Colletotrichum gloeosporioides*, *Colletotrichum* spp., *Curvularia* spp., *Cylindrocladium* spp., *Dolabra nepheliae*, *Fusarium* spp., *Gliocephalotrichum bulbilium*, *Lasmenia* spp., *Phomopsis* spp., and *Septoria* spp. Pathogenicity tests are in progress under laboratory and greenhouse conditions, using seedlings and detached fruit. PCR amplification of the rDNA ITS region and the beta-tubulin gene will complement morphological identification of fungi.

Use of bio-enhanced organic mulches for integrated management of nutsedge in tomato

Y. SHABANA (2), E. Rosskopf (5), R. Charudattan (2), A. Abou Tabl (1), W. Klassen (3), J. Morales-Payan (4)
(1) Mansoura University, El-Mansoura, Egypt; (2) University of Florida, Gainesville, FL, USA; (3) University of Florida, Homestead, FL; (4) University of Puerto Rico-Mayaguez, Mayaguez, PR; (5) USDA, ARS, USHRL, Fort Pierce, FL, USA
Phytopathology 100:S176

Purple and yellow nutsedges (*Cyperus rotundus* and *C. esculentus*, respectively) are among the world's most problematic weeds that impact virtually every horticultural crop grown in Florida and the Caribbean. As an alternative to conventional methods of control that includes the use of soil fumigation with methyl bromide, we tested nine hay mulches (shoot straw of bahiagrass, cogongrass, cowpea, millet, yellow nutsedge, sorghum Sudangrass, sunnhemp, and rye) and three green mulches (shoot biomass of cowpea, millet, and sorghum Sudangrass) as a means to suppress nutsedge growth in a raised-bed tomato (cv. Tygress) field. In addition, two fungus-infested cogongrass hays (infested with the nutsedge pathogen *Dactylaria higginsii* [Dh] or the saprophytic fungus *Trichoderma* sp. [Tri]), and two plastic mulches (black and infra-red transmissible [IRT]) were tested. The black plastic mulch and the Dh-infested cogongrass mulch consistently reduced nutsedge emergence and growth more than the other organic mulches and the IRT plastic mulch. Among the organic mulches, cogongrass infested with Dh or Tri and cowpea, sunnhemp, Bahiagrass, and cogongrass provided the highest levels of nutsedge suppression. No disease symptoms developed on nutsedge plants when Dh- or Tri-infested cogongrass was used as the mulch. Both plastic mulches (black and IRT) and Tri-infested cogongrass enhanced tomato yield and the proportion of larger fruits. The highest yield of extra large tomatoes per plant was obtained when these mulches were applied.

Laurel wilt of avocado: Management and mitigation research in Florida

J. A. SMITH (1), R. C. Ploetz (2), T. J. Dreaden (1)
(1) School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA; (2) Tropical Research and Education Center, University of Florida, Homestead, FL, USA
Phytopathology 100:S176

Laurel wilt, caused by the fungus *Raffaelea lauricola* and transmitted by the exotic redbay ambrosia beetle, *Xyleborus glabratus*, threatens the U.S. avocado industry with elimination if drastic measures are not taken in the near future. Since its introduction in Georgia approximately 6 years ago, the disease has spread on several hosts in the Lauraceae on the southeastern coastal plain and now looms only 100 miles from commercial avocado groves

(7,500 acres worth $34 million/yr) in Miami-Dade County, FL. Within the last 2 years, door-yard avocadoes have been rapidly killed and serve as a source of inoculum for the epidemic. Current research efforts include: examining extant avocado germplasm for resistance; using a taxon-specific real-time PCR technique to diagnose the pathogen and identify it in screening and epidemiology studies; and fungicide efficacy trials. The results from these studies will be presented and future work will be discussed. Laurel wilt threatens avocado production worldwide. Thus, we will address its potential impact and preventing its movement to new areas.

Combating the loss of red bay and other native species to laurel wilt
J. A. SMITH (3), M. A. Hughes (1), C. Anderson (3), R. C. Ploetz (4), A. E. Mayfield (2)
(1) Department of Plant Pathology, University of Florida, Gainesville, FL, USA; (2) Florida DACS Division of Forestry, Gainesville, FL, USA; (3) School of Forest Resources and Conservation, University of Florida, Gainesville, FL, USA; (4) Tropical Research and Education Center, University of Florida, Gainesville, FL, USA
Phytopathology 100:S177

Laurel wilt, caused by *Raffaelea lauricola*, currently threatens all native and some exotic species in the Lauraceae in the United States. Since 2003, the disease has devastated native stands of redbay, *Persea borbonia*, and threatens several other taxa in the family, including avocado. Two natives, pondspice (*Litsea aestivalis*) and pondberry (*Lindera melissifolia*), which are on state endangered and federal critically endangered lists, respectively, face extinction. Despite sanitation and other efforts to slow the movement of laurel wilt, it continues to move to new areas every year, largely due to the efficiency of the disease vector, the exotic redbay ambrosia beetle (*Xyleborus glabratus*). There are many gaps in our current state of knowledge about the biology of the disease, and several studies are underway. Current research has focused on: protecting existing trees via fungicides; identifying and utilizing putative resistance in redbay and avocado; elucidating the disease's epidemiology and host range; and determining to what extent genetic and pathogenic variation exist in the *R. lauricola* population. An update on the results from these studies will be given and future research needs will be discussed.

Effect of acibenzolar-S-methyl on bacterial leaf spot of shrub roses caused by a *Xanthomonas* sp.
G. E. VALLAD (1)
(1) Gulf Coast REC, University of Florida, Wimauma, FL, USA
Phytopathology 100:S177

A bacterial leaf spot was recently identified on shrub rose varieties 'Knockout' and 'Double Knockout' caused by a *Xanthomonas* sp. and can be problematic during vegetative propagation and nursery production. Acibenzolar-S-methyl, the active ingredient of Actigard (Syngenta, Greensboro, NC), is an elicitor of plant defenses that has demonstrated efficacy in the control of several bacterial diseases of vegetable crops. Greenhouse and nursery trials were established to test the effect of Actigard on the severity of bacterial leaf spot on 'Knockout' and 'Double knockout' roses. While lower rates of 0.25 to 0.5 oz of Actigard per 100 gallons were effective at reducing disease severity on potted roses and liners, higher rates of 0.75 to 1.0 oz gave the best results. Multiple applications of Actigard (1.0 oz/100 gal) prior to disease development improved bacterial leaf spot control over single applications. Results demonstrate the potential to use Actigard for disease management on ornamental and nursery species.

Effect of acibenzolar-S-methyl on the management of early blight and target spot of tomato
G. E. VALLAD (1)
(1) Gulf Coast REC, University of Florida, Wimauma, FL, USA
Phytopathology 100:S177

Acibenzolar-S-methyl, the active ingredient of Actigard (Syngenta, Greensboro, NC), is an elicitor of plant defenses. While labeled for tomato, usage is currently limited to the control of bacterial leaf spot (*Xanthomonas* spp.) and bacterial speck (*Pseudomonas syringae* pv. *tomato*). In 2008, two field trials assessed the performance of Actigard (8 weekly applications at 0.75 oz per acre) when integrated into a standard spray program that included weekly applications of copper sulfate (2.1 lbs a.i. per acre) mixed with either mancozeb (1.5 lbs a.i. per acre) or chlorothalonil (1.5 lbs a.i. per acre). The addition of Actigard reduced the severity of early blight (*Alternaria solani*) and target spot (*Corynespora cassiicola*) by 22 to 44% over the standard spray program alone, and by 31 to 60% compared to the non-treated plots. In the spring trial, plots treated with Actigard yielded 336 more cartons (25 lbs) of marketable tomatoes per an acre than those receiving the standard alone, and 1,179 cartons more per an acre than the non-treated plots. No yield improvement was observed in the fall trial, due to the development of disease in the late season. Results demonstrate the benefit of including Actigard as part of an overall spray program to manage common foliar diseases caused by bacterial and fungal pathogens of tomato.

***Salmonella* outbreaks associated with vegetables: How high is the risk?**
A. VAN BRUGGEN (1)
(1) Emerging Pathogens Institute and Plant Pathology Department, IFAS, University of Florida, Gainesville, FL, USA
Phytopathology 100:S177

Gastro-enteritis outbreaks increased in the 1990s and have remained steady since then. Most outbreaks have been associated with seafood, but most individual cases with vegetables and fruits (38% of all cases). *Salmonella enterica* is the most common pathogen involved in outbreaks associated with vegetables. Several *Salmonella* outbreaks were traced back to contaminated tomatoes. *Salmonella enterica* is very versatile: there are more than 2500 serovars, which occur in various environments, including many plant and animal species. The main reservoirs are the intestines of birds, pigs, cattle, wild mammals and reptiles, but they are also harbored by protozoa, earthworms, nematodes and snails. They can multiply in the rhizosphere of various plants and occur on plant surfaces as well as in the endosphere. Because of the human as well as economic costs associated with *Salmonella* outbreaks, it is important to study the risk of an outbreak to occur. However, there are different kinds of risk: calculated probabilities as well as perceived risks. These last risks are concerns voiced by consumers on a comparative scale. Among various safety concerns, microbiological risks are ranked high, due to some knowledge and experience and the feeling of not being able to control exposure. Perceived risks do not necessarily coincide with calculated probabilities, but may be more influential in terms of the response to an outbreak. Quantitative microbial risk assessment consists of several steps: hazard identification and characterization, exposure assessment and risk characterization. In a project on risk assessment of enteric pathogens in the vegetable production chain, we limited ourselves to exposure assessment through lettuce contaminated from manure and soil. The occurrence and survival of enteropathogens in cattle manure were primarily determined by the feed given to the cattle: low-fiber feed resulted in more shedding and longer survival in the low-fiber and low-pH manure. Other risk factors were low numbers of nonpathogenic coliform bacteria and high dissolved organic carbon contents. Constant temperatures and low oxygen levels also contributed to long survival times in manure. Survival times in soil were negatively correlated to microbial diversity and positively to dissolved organic carbon contents. A probabilistic exposure model for *E. coli* O157:H7 resulted in a relatively low probability of about 1 contaminated head in 10,000 lettuce heads. The risk can best be reduced at the beginning of the production chain, the cattle farm. There are several quantitative risk assessment models for *Salmonella*, but most of them are for animal products, except for one model for almond contamination and on for vegetable contamination from irrigation water. No risk model was found for tomato production and processing. In a tomato safety research workshop, research needs were identified, but control at the source of the chain was not mentioned while this was the crucial factor in our lettuce risk model. Risk models based on calculated probabilities could be used to influence perceived risks by the general public.

STAR-D: The NPDN accreditation program for diagnostic laboratories
A. B. VITORELI (1), C. L. Harmon (1)
(1) University of Florida, Gainesville, FL, USA
Phytopathology 100:S177

The Food and Agriculture Defense Initiative was established in 2002 to enable the United States Department of Agriculture to develop a network linking plant and animal disease diagnostic facilities across the USA. The National Plant Diagnostic Network (NPDN) is the plant disease component of this network. The mission of the NPDN requires quick and accurate diagnosis of high consequence plant pathogens, weeds and insect pests that threaten national security; communication of such information response authorities; the ability to scale up and manage sample surge as needed; and diagnostic data security. To accomplish these objectives, the NPDN relies on diagnostic data generated by laboratories in Land-Grant Universities, State Departments of Agriculture, and USDA-APHIS. Traditionally, these laboratories have provided diagnostic services at the State or regional levels at a high level of competence. However, to accomplish the national objectives listed above, a standardized approach to diagnosis is required, particularly if the diagnosis has regulatory implications. The NPDN System for True, Accurate, and Reliable Diagnostics (STAR-D) has been developed to enable participating laboratories to meet standards of quality for laboratory management, facilities, equipment, and trained personnel. The ISO 17025 Quality Standard can be adapted to testing done by NPDN diagnostic laboratories by providing a basis for a quality system to meet the needs of the NPDN STAR-D.

Levels of P in Areca catechu leaves following phosphorous acid application through adventitious roots

G. C. WALL (1)
(1) University of Guam, Mangilao, GU 96923, USA
Phytopathology 100:S178

Bud rot disease of betel nut (Areca catechu L) has been shown to be caused by Phytophthora palmivora. Fosphite, or phosphorous acid, is recommended for the control of Phytophthora, applied by injection to the trunk. After finding evidence of damage associated with injection sites in the trunks of betel nut trees, a decision was made to look for other ways of applying the fungicide in order to avoid damaging the trees. A paired t test was devised to study the effect of applying phosphorous acid solution via adventitious roots of betel nut trees. A group of mature trees was sampled pre- and post-application. Levels of P were determined from leaf samples collected from each frond per tree. There were 12 pairs of trees in the study; one set of trees was treated with the recommended rate (applied by absorption through an adventitious root) and half were controls, treated only with sterile distilled water also via one adventitious root. After the appropriate statistical analysis (NCSS, Kaysville, UT), differences were found in the level of P in the leaf samples according to treatment. Control trees had higher levels of P in their leaf tissue compared to trees given phosphorous acid. The underlying hypothesis was that application of the fungicide via adventitious roots of trees would result in a systemic distribution of the fungicide throughout the tree. It was expected that all leaf samples from treated trees would show higher levels of P compared to untreated controls. Surprisingly, P levels were lower in treated trees, yet there was no difference between fronds, suggesting an even effect throughout treated trees. No explanation is known at this time for the reduced P levels observed after treatment; however, results were consistent enough to yield highly significant differences statistically.

Epidemiology of soybean rust (*Phakopsora pachyrhizi*) in soybean (*Glycine max*) sentinel plots in Florida

H. M. YOUNG (2), J. J. Marois (2), D. L. Wright (2), D. F. Narvaez (1), G. K. O'Brien (2)
(1) Monsanto, St. Louis, MO, USA; (2) University of Florida, NFREC, Quincy, FL, USA
Phytopathology 100:S178

The overwintering of soybean rust (SBR) in the Southeastern United States has been variable due to weather conditions which may influence disease incidence and severity in the major soybean producing regions of the Midwest, making it important to understand the epidemiology of the pathogen in Florida. This study examined the incidence and severity of SBR in relation to prevailing weather data, growth stage, and maturity group (MGIII, MGV, MGVII) in 15 m square soybean plots across the Panhandle of Florida from 2005 through 2008. Of the three maturity groups, the MGIII soybean became infected first the least often. Plots became infected first at growth stage R4 (full pod) or later. On average, plots became infected 40 days earlier in 2008 than 2005. Precipitation was the principle factor affecting disease progress, where disease increased rapidly after rain events and was suppressed during dry periods. The area under the disease progress curves (AUDPC) for incidence and severity was the lowest in 2007, most likely due to dry conditions. In 2008, there was a significant increase in disease incidence and severity as reflected in the AUDPC. This can be attributed in part to the occurrence of Tropical Storm Fay, which deposited up to 290 mm of water in the plot locations during the third week of August. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this pathogen.

Effect of rhizobacteria, acibenzolar and silicon on bacterial spot of tomato

S. ZHANG (1), T. L. White (1), W. Klassen (1), M. C. Martinez (1)
(1) Tropical REC, University of Florida, IFAS, Homestead, FL, USA
Phytopathology 100:S178

Bacterial spot, caused by *Xanthomonas perforans*, is one of the most economically important diseases of tomato in Florida and other tomato grown areas worldwide. Chemical controls have been only partially effective due to the wet and warm climate in Florida and the development of resistance in populations of this bacterial pathogen. It is imperative that practical alternative strategies be developed to sustain the production of tomatoes. Greenhouse and field trials have been conducted to investigate the effect of plant growth-promoting rhizobacteria (PGPR), acibenzolar-S-methyl (ASM) and silicon nutrient on bacterial spot of tomato. In the greenhouse, eight bacilli PGPR strains were evaluated on two cultivars of tomato (FL47R and Tygress). Tomato seeds were sown into pro-mix in 128-cell Styrofoam flats and grown for 1–2 weeks when solutions of PGPR, Actigard 50WG (ASM) and silicic acid were applied weekly as soil drenches. Tomato seedlings were transplanted into 4-inch pots containing potting mix after 3–4 soil drenches, and inoculated by foliar spray with suspensions of *X. perforans* at 1×10^8 CFU/ml. Results indicated that PGPR strain SE76 and INR7 significantly ($P < 0.05$) reduced disease severity of bacterial spot on both tomato cultivars compared to the nontreated control. SE52 on cv. FL47R and SE34, IN937a and IN937b on cv. Tygress each had a significant effect on disease reduction. In the first field trial on tomato cv. FL47R, Actigard 50 WG at 30 mg/l significantly suppressed bacterial spot rated at 8, 9, 10 and 11 weeks after transplanting, whereas silicic acid at 0.15 and 1.5 mM did so only at 8 weeks. In another field experiment, the same eight PGPR strains and Actigard 50WG at 30 and 3 mg/l were tested on tomato cv. Tygress. Significant disease reduction was observed on tomato plants treated with PGPR strains IN937a and IN937b 3 weeks after transplanting.

2009 North Central Division Meeting Abstracts
Symposia Presentations

Abstracts submitted for presentation at the Symposia Presentations held at the 2009 North Central Division meeting in Ames, Iowa, June 21–23, 2009. The abstracts are arranged in order of presentation.

Implications of Climate Change on Plant Pathogens

Climate impacts on agriculture: Implications for crop management
J. L. HATFIELD (1)
(1) National Soil Tilth Laboratory, Ames, IA, USA
Phytopathology 100:S179

Climate impacts on agriculture can be either direct or indirect in terms of affecting production or quality of the product. The direct impacts result from temperature, precipitation, or CO_2 effects on crop growth and development while the indirect impacts result from the climatic impacts on weed, insect, or disease populations which in turn affect crop production. Climate has changed, is changing, and will continue to change; however, the current state of the climate relative to agriculture is one in which there is increasing variability in temperature and precipitation and rising CO_2 concentrations. Increasing temperatures hasten plant development and can lead to plant stress when there are limited soil water supplies. One aspect of rising CO_2 concentrations is increased water use efficiency; however, this may not overcome plant stress induced by lack of soil water recharge by variable precipitation. Variations in climatic parameters will affect growth and development of weeds which will make weed management an increasing challenge. Changing temperature and precipitation patterns will affect the overwintering of insects and diseases and increase the range of pests. Development of management strategies to increase agricultural production will have to consider all aspects of climate on the agricultural systems and where the opportunities exist for improved efficiency in crop production and potential for enhanced crop protection strategies.

Climate change and food security
L. ZISKA (1)
(1) USDA Crop Systems and Global Change Lab, Beltsville, MD, USA
Phytopathology 100:S179

Documented and projected changes in atmospheric carbon dioxide are likely to alter agricultural productivity in two ways: directly, by supplying additional carbon for photosynthesis and growth; and, indirectly, by altering climate, specifically surface temperatures and precipitation. In this overview on the impact of carbon dioxide and climate change on food security, I will present data from a number of sources that document the likely changes in temperature, temperature and carbon dioxide and water availability on crop quality and production, and identify other biological interactions with pests, weeds, and diseases. In addition, I will discuss possible opportunities, focusing on exploitation of genetic and intra-specific variability within plant germplasm as a possible means to maintain agricultural production in the future.

Forecasting weather and climate for plant disease models: A western perspective
L. COOP and the Western Weather Workgroup Integrated Plant Protection Center (1)
(1) Oregon State University, Corvallis, OR, USA
Phytopathology 100:S179

Input requirements for many plant disease models currently challenge our ability to incorporate short and long term climate change effects. In the Western US, these challenges supplement ones incited by the mountain and coastal influenced terrain. The Western Weather Workgroup has addressed some of these needs via a series of meetings, grant projects, and on-farm field trials. Some helpful technologies that address the problems of scale in disease forecasting that we are using include PRISM climate mapping, mesoscale weather forecasts, online error analysis, ingest of real time public and private weather observations, and new methods for downscaling IPCC climate change model projections. A proposed Swiss Needlecast model for PNW forests can benefit from these climate change model projections, and so can degree-day model forecasts by using modified climate "normals". Many of the technologies presented have been incorporated into a nationally focused website, http://uspest.org/wea, which addresses several IPM and plant biosecurity needs.

Modeling plant disease in a changing climate
J. RUSSO (1)
(1) ZedX, Inc., Bellefonte, PA, USA
Phytopathology 100:S179

Environment, along with host and pathogen, are the long-recognized key components for disease development. A changing climate impacts the environment component and, by their triangular relationship, host and pathogen. The challenge to plant epidemiological modelers is to incorporate the appropriate weather variables for quantifying the impact of a changing climate on disease development. Furthermore, modelers must be sensitive to the spatial and temporal scales represented by weather variable data and the uncertainty associated with the data in predicted disease behavior. Lastly, even after accounting for scale and uncertainty, the modeling of plant disease in a changing climate must be evaluated in the context of an ecosystem.

Nematode Pests of the North Central Region

Insights into the mode of action of cyst nematode effector proteins
T. HEWEZI (1), T. J. Baum (1)
(1) Iowa State University, Department of Plant Pathology, Ames, IA, USA
Phytopathology 100:S179

Plant-parasitic cyst nematodes secrete proteinaceous effectors, which play the central role in host infection and formation of their feeding sites. The majority of these proteins are novel and their putative functions can not be assigned due to the absence of significant sequence similarities to known proteins in sequence databases. Elucidation of the mechanism of action of these effectors is an absolute necessity for engineering resistance to these damaging plant pests. Remarkable progress has been made recently in understanding the mode of action of cyst nematode effectors. Two effectors targeting different subcellular compartments have been functionally characterized. The first effector is a cellulose binding protein (CBP) that acts in cell wall modification.

Transgenic plants expressing CBP revealed its vital role in mediating plant susceptibility to cyst nematode infection. We identified pectin methylesterase 3 (PME3) as a strong and specific interactor of CBP. Our data indicate that CBP interacts with PME3 thereby activating and potentially targeting this enzyme to modulate the properties of the cell wall via modification of pectin, and subsequently affecting plant growth and pathogen susceptibility. The second effector is a cytoplasmic protein, which we term 10A06. 10A06 was found to affect plant morphology and nematode susceptibility when expressed *in planta*. 10A06 specifically interacts with a plant spermidine synthase (SPDS). Our results collectively indicate that 10A06 functions in modulating polyamine signaling to promote plant susceptibility.

Plant-parasitic nematodes in midwestern corn fields
T. A. JACKSON (1)
(1) University of Nebraska, Lincoln, NE, USA
Phytopathology 100:S180

Plant parasitic nematodes have historically been a costly challenge for corn production in some areas, but some common cropping practices helped to mitigate their impacts. During recent years, producers have utilized more pyrethroid insecticides and transgenic insect resistant corn hybrids, neither of which is as effective against nematodes as the organosphosphate and carbamate soil insecticides used in the past. The recent increase in the incidence of damage caused by nematodes, particularly in the Midwest has led to questions about the prevalence of nematodes in corn fields and the potential for further injury. In 2006, a preliminary survey, funded by Syngenta Seed Care, of corn fields in Nebraska was initiated and expanded to include other states in the corn belt during 2007. Three fields were arbitrarily selected to represent each county with more than 20,000 acres of corn. Both soil and root samples were collected from the fields and submitted to one of six laboratories, five university and one private laboratory, for analysis. Results of the analyses indicated that plant parasitic nematodes were present in practically every field to varying degrees, with some population densities exceeding historic estimates of damage thresholds. Results also varied markedly by state, indicating that the efficiency of the nematode extraction procedures varied between laboratories. These results indicate that there is the potential for yield loss in corn caused by nematodes warranting further research.

***Pratylenchus penetrans* is a common and persistent pathogen of potato in the north central region**
A. E. MACGUIDWIN (1)
(1) University of Wisconsin-Madison, Madison, WI, USA
Phytopathology 100:S180

Root lesion nematodes, *Pratylenchus* spp., are the most common nematode problem in the North Central region. *Pratylenchus penetrans* is the species most damaging to a wide range of crops including potato. Surveys showed 9 of 102 potato fields in Wisconsin surpassed the threshold for nematode damage (200 per 100 cc soil). The same number of fields surpassed the damage threshold for *Verticillium dahliae* (10 propagules per gram soil). The majority of the remaining fields were infested with subthreshold densities of both pathogens and at risk for the potato early dying disease (PED) caused by the interaction of *V. dahliae* and *P. penetrans*. The potential for PED in the surveyed fields was verified by a bioassay and corroborated our laboratory research showing the interaction of these two pathogens. Historical data shows that population densities of *P. penetrans* have increased in Wisconsin over the last twenty years. One of the factors likely to have contributed to an increase in *P. penetrans* is a change in crop rotations. Rotation crops vary in their host status for reproduction by *P. penetrans*, but also important are root system characteristics that impact the quantity and quality of dead root fragments serving as reservoirs of nematode inoculum for the next crop. Our analysis of many historical data sets showed that a significant proportion of a *P. penetrans* population survives in detached root fragments when live hosts are not available. Growers recognize the importance of *P. penetrans* to potato, but are only beginning to appreciate the impact of *P. penetrans* on other crops and the role that crop rotation plays in the root lesion nematode disease of potato.

Implications of Plant Diseases in Biofuel Production

Plant disease in *Miscanthus* and other cellulosic biomass crops
E. HEATON (1)
(1) Iowa State University, Dept. of Agronomy, Ames, IA, USA
Phytopathology 100:S180

Cellulosic biomass crops are slated for production on roughly 30 million acres of U.S. farmland in the next 20 years but very little is known about the implications plant disease might have for these crops. To date, the acreage of dedicated energy crops is small, and disease issues have yet to cause concern. What issues might we expect? What has been observed in small stands? Reproductive growth is not important in cellulosic crops, therefore chief concerns are expected to be foliar blights that reduce carbon assimilation, and root and stalk rots that reduce harvestable yield. In the Midwest, sorghum (*Sorghum bicolor*), switchgrass (*Panicum virgatum*) and Miscanthus (*Miscanthus* × *giganteus*) are the leading herbaceous candidate biomass crops. Sorghum is a familiar crop now being bred for dedicated biomass production. Diseases currently problematic for forage sorghum including downy mildew, (*Peronosclerospora sorghi*), *Fusarium* spp. and Anthracnose (*Colletotricum graminicola*) will become increasingly important for biomass sorghum. Switchgrass has been for studied as a biomass crop for decades, but still little is known about its pests and pathogens. Principle diseases include rusts, smuts, root rots and *Panicum mosaic virus*. Generally, diseases have not caused major yield reductions and susceptibility varies among cultivars. Miscanthus is still new to the US but has been studied and used in Europe for nearly 20 years. A relative of sugarcane, *M.* × *giganteus* is sterile and currently planted from rhizome pieces, leading to clonal fields with little or no genetic variation over large areas. Even so, no economically important diseases have emerged in *M.* × *giganteus*. In Europe, *Fusarium* rots and *Barley yellow dwarf virus* have been reported. A disease survey of *M.* × *giganteus* recently conducted across the Midwest has observed 1 virus, 5 fungal diseases and 10 different genera of plant parasitic nematodes present in fields.

Impact of diseases on biomass productivity in switchgrass
G. P. MUNKVOLD (1)
(1) Iowa State University, Dept. of Plant Pathology, Ames, IA, USA
Phytopathology 100:S180

Switchgrass (*Panicum virgatum*) is widely considered as a preferred feedstock for lignocellulosic ethanol production, which could lead to a significant increase in area planted to this crop. Current switchgrass production is dominated by a few cultivars that have been developed for site adaptation but not specifically for disease resistance. Increased density of switchgrass in the landscape may exacerbate existing disease problems, which could present a significant obstacle to biomass productivity. The diseases with the greatest potential to suppress biomass yield are switchgrass smut, caused by *Tilletia maclaganii*, and switchgrass rust, *Puccinia emaculata*. The smut disease results in stunting, premature flowering, and replacement of seeds by fungal sori; it has been reported from several states spanning from Kansas to New York, and likely occurs elsewhere in the U.S. Studies in Iowa indicated that the disease occurred in over 50% of the area planted to switchgrass, and that it reaches high levels of incidence (up to 70%) in older stands. Smut incidence, stand density, and yield were determined in 10 fields differing in disease incidence (from 0.7 to 55.4%, mean 26%). Mean biomass/tiller was reduced by 38 to 82% in diseased tillers compared to healthy tillers. Yield loss estimates ranged from 1.7 to 40.1% among the fields. Disease incidence and yield loss had a linear relationship (R^2 = 0.95) and, based on regression modeling, yield loss for all sampled fields was estimated at 17.0%. Economically viable switchgrass production will require strategies to reduce the impact of smut and rust. Cultivar development is the most promising approach, and recent efforts include selection for *P. emaculata* resistance, but resistance to *T. maclaganii* has not been identified consistently in any current cultivars, and sources of resistance for use in breeding are not yet evident.

Concentration of *Fusarium* toxins in naturally contaminated corn and corn processing co-products derived from ethanol production
A. W. Schaafsma (1), V. LIMAY-RIOS (1), and J. D. Miller (2)
(1) Ridgetown College, University of Guelph, Ridgetown, Ontario, Canada;
(2) Carleton University, Ottawa, Ontario, Canada
Phytopathology 100:S180

In north temperate areas such as Ontario, contamination by toxins from *Fusarium graminearum*, particularly deoxynivalenol (DON) and zearalenone, is common. Fumonisin contribution has been modest by comparison with other corn-producing areas. In this study, three matrices [corn meal, distiller's dried grains with solubles (DDGS), and condensed distiller's soluble (CDS)] were sampled in sequence from a continuous dry milling processing plant for the determination of mass balance of DON. LC-MS/MS was used as a confirmatory method for determination of DON and other *Fusarium* toxins. DON concentrations in the CDS and the final DDGS co-product were significantly higher ($P \leq 0.01$) than in the starting material (corn grain). Toxin concentration increased by a factor of 3 on a dry weight basis in DDGS compared to the starting corn, and by 4 in CDS. Mean concentration of DON in CDS was four times higher (7.1 mg kg^{-1}) than in corn grains (1.8 mg kg^{-1}) and 1.4 times higher than in DDGS (5.24 mg kg^{-1}). Mass balance calculations show that CDS is the main source of contamination of DON comprising ca. 70% of the toxin found in the final product (DDGS). Most DON (87%) was

accounted for by this analysis. The presence of mycotoxins in DDGS and CDS affects their utility as animal feed supplements. Our data indicate that concentrations in the grain corn entering ethanol plants should be close to the dietary values recommended for swine in Canada and the United States for DON (1 mg kg^{-1}). Aside from DON, small amounts of acetyldeoxynivalenol, DON glucoside and zearalenone were found in corn, DDGS and CDS. Unlike the situation for DON, the DON glucoside was not concentrated into DDGS and CDS. This indicates that some DON glucoside may have been hydrolyzed during the fermentation process.

Microbial characterization of distillers wet grains: Results and challenges
M. LEHMAN (1)
(1) USDA-ARS North Central Agronomy Research Laboratory, Brookings, SD, USA
Phytopathology 100:S181

Distillers grains are co-produced with ethanol and carbon dioxide during the production of fuel ethanol from the dry milling and fermentation of corn grain, yet there is little basic microbiological information on these materials. We have characterized the microbiology of distillers wet grains (DWG) over a nine-day period following their production at an industrial fuel ethanol plant. This freshly-produced DWG had a pH of about 4.4, a moisture content of about 53.5% (wet weight basis), and 4×10^5 total yeast cells/dry g, of which about 0.1% were viable. Total bacteria cells were initially below detection limits (ca. 10^6 cells/dry g) and then were estimated to be ~ 5×10^7 cells/dry g during the first four days following production. Culturable aerobic heterotrophic organisms (fungi plus bacteria) ranged between 10^4 and 10^5 CFU/dry g during the initial four day period and lactic-acid bacteria (LAB) increased from 36 to 10^3 CFU/dry g over this same period. After nine days, total viable bacteria and yeasts/molds topped 10^8 CFU/dry g and LAB approached 10^6 CFU/dry g. Community phospholipid fatty acid analysis (PLFA) yielded limited data, but indicated a stable microbial community over the first four days of storage. Thirteen morphologically-distinct isolates were recovered of which ten were yeasts and molds from six different genera, two were strains of the lactic acid-producing *Pediococcus pentosaceus*, and only one was an aerobic heterotrophic bacteria, *Micrococcus luteus*. The microbiology of DWG is fundamental to assessment of spoilage, deleterious effects (e.g., toxins), or beneficial effects (e.g., probiotics) in its use as feed or in alternative applications. Significant challenges are encountered when applying culture-independent analyses (DNA-based, PLFA, total protein, and direct observation techniques) to characterize the microbiology of wet distillers grains.

Potential Crop Biosecurity Risks that Threaten Agriculture in the North Central Region: Staying Ahead of the Curve

Disease threats to natural and agricultural plant systems: Think locally, act globally
J. STACK (1)
(1) Kansas State University, Manhattan, KS, USA
Phytopathology 100:S181

Plant health is the foundation for human health and wellbeing. Plant-based agricultural systems are critical to the economies of many states in the Midwest and Great Plains regions of the United States and the exports from these regions contribute to global food security. Recurring and emerging diseases pose direct and indirect threats to sustainability of plant, animal, and human systems. Comprehensive plant biosecurity plans are necessary prerequisites to sustainable plant health in the face of the long list of general and specific threats that results from global trade, climate change, population growth, and biocrime. A plant biosecurity strategy that minimizes the impacts from plant diseases without compromising production efficiency and trade is essential. Among the challenges to plant biosecurity are: 1) the ability to accurately identify and prioritize pathogen threats and plant system vulnerabilities, 2) the ability to develop preparedness plans that are strong enough to protect plant systems from identified threats while robust enough to protect against unanticipated emerging disease threats, and 3) the development of resilience in natural and agricultural plant systems. We need to think locally (e.g., develop strong plant biosecurity plans and don't import uninspected plants or plant products) and act globally (e.g., support national and international phytosanitary regulations and don't export uninspected plants or plant products). While the threats from bioterrorism are often overstated, the threats from accidental introductions as a result of global trade in plants and plant products are often understated. The large number of existing disease threats to Midwest and Great Plains plant systems, the potential for newly emerging yet unknown disease threats, and our poor ability to accurately prioritize those threats requires a more general approach to plant biosecurity. The uncertainty associated with the processes of threat identification, vulnerability assessment, and impact prediction should be cause for concern.

Regional and national efforts to enhance detection and diagnostics
R. HAMMERSCHMIDT (1)
(1) Michigan State University, East Lansing, MI, USA
Phytopathology 100:S181

The threat of accidental and intentional introductions of new pathogens and pests along with the potential for re-emergence of older disease/pest problems illustrates the need for enhanced capacity diagnostics and detection. Two programs, the USDA-CSREES sponsored National Plant Diagnostic Network (NPDN) and ipmPIPE (Pest Information Platform for Extension Education), which is sponsored by several USDA agencies and other private and public groups, will be used to describe recent efforts to improve diagnostic and detection capacity. Since its inception in 2002, the NPDN has, for example: enhanced diagnostic capacity at land grant diagnostics labs; provided diagnostic training for new disease problems; assisted in the development of standard operating procedures for diagnostics; developed and deployed first detector training programs; and conducted disease detection and diagnostic exercises. Through these efforts, there has been an improvement in our ability to detect and diagnose as well as enhanced communication and cooperation among the land grant university diagnosticians, state departments of agriculture and USDA-APHIS. The ipmPIPE is a national warning system to help growers protect their crops from the diseases and pests. The program was initiated with funding from USDA and the soybean industry to assist in early detection and diagnosis of Asian soybean rust. There are now four additional ipmPIPE programs: soybean aphid, legumes, cucurbit downy mildew, and pecan nut casebearer. In each of the ipmPIPE programs, field observations and sampling are conducted by the land grant university in each state. Samples are examined by university NPDN labs or state specialists, and the results are entered into electronic databases. There is a public website that is available for use by growers and others for obtaining current information on disease/pest spread and management recommendations. For more information visit: www.NPDN.org and www.ipmpipe.org.

The role of the seed industry in crop biosecurity
W. E. DOLEZAL (1)
(1) Pioneer Hi-Bred International, Inc., Johnston, IA, USA
Phytopathology 100:S181

The North Central Region of the United States is a rich agricultural production region for several major commodities, including corn, soybean, wheat and sunflower. It is also a major region for seed production, especially for corn and soybean. The establishment and continued funding of the National Plant Diagnostic Network, with its regional networks and the Soybean, Legume and Soybean Aphid ipm-PIPE programs have greatly aided in the accurate identification and monitoring of major economic pests which threaten crops in the North Central Region. Efforts for building low cost, true partnerships with seed industry personnel in existing federal and state pest monitoring programs, plus a pilot public/private collaborative effort in monitoring *Puccinia polysora* will be discussed.

Meeting the challenges of U.S. crop biosecurity: Pre- and post threat introduction
F. W. NUTTER, JR. (1), N. Holah (1), N. Van Rij (2), D. Wright (3), J. Marois (3)
(1) Iowa State University, Ames, IA, USA; (2) Cedara Department of Agriculture, Pietermaritzburg, South Africa; (3) University of Florida, Quincy, FL, USA
Phytopathology 100:S181

The global monitoring of exotic biotic plant pathogens, prior to their introduction into the U.S. by natural, accidental, or deliberate means, remains a key challenge in the effort to safeguard our nation's agricultural biosecurity. Remote sensing, GPS and GIS technologies are now being integrated and utilized successfully to identify specific plant pathogens. This new paradigm replaces less successful attempts to find and apply unique spectral signatures for pathogen identification. Pathogen-specific temporal and spatial signatures for Asian soybean rust and Cercospora leaf spot epidemics affecting soybean crops grown in South Africa, Argentina, and the U.S. were extracted from high resolution (<1.0 m^2 per pixel) satellite images obtained by commercial satellites. Such approaches offer the means to detect and correctly identify biotic threats prior to (and after) introduction into the US, thereby serving as both an early (pre-introduction) warning system and as a tool for post-introduction response.

2009 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in Ames, Iowa, June 21–23, 2009. The abstracts are arranged alphabetically, by first author's name.

Genetic diversity of *Colletotrichum coccodes* vegetative compatibility groups using Fluorescent Amplified Fragment Length Polymorphism markers

K. M. ALANANBEH (1), N. Gudmestad (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 100:S182

Colletotrichum coccodes (Wallr.) Hughes, is a cosmopolitan pathogen that has wide distribution and host range. *C. coccodes* is an imperfect fungus and vegetative compatibility serves as a means of genetic exchange and is useful for measuring genotypic diversity. Seven vegetative compatibility groups (VCG's) have been identified for this fungus using nitrate nit mutants. Vegetative incompatibility (*vic*) alleles present among continental populations prevents anastomosis from occurring among these populations thereby limiting VCG as a method to evaluate diversity of the global population. The main objective of this study was to study the genetic diversity of the VCG's among the North American, European, and Middle Eastern isolates of *C. coccodes* using Fluorescent Amplified Fragment Length Polymorphism (AFLP) markers to obtain a better understanding of the genetic diversity of the global population. A total of 526 isolates of *C. coccodes* were used in this study, 311 were from North America (NA), 183 from Middle East (Israel), and 32 isolates from Scotland. Three AFLP primer sets were used to generate amplified fragments. The *C. coccodes* isolates were compared with 62 isolates previously studied. All DNA fragments within the range of 100 to 620 bp were scored manually for the three primer sets. The bands were scored for presence or absence (1 = presence or 0 = absence). Binomial data was used to create a similarity matrix using the WINDIST application of the WINBOOT program and the DICE similarity coefficient. Analysis of the first primer set showed that the NA–isolates were assigned to VCG's 1, 2, 3, 4, 5, and 6. This is consistent with previous findings. Israeli isolates were assigned to VCG's 2 and 5, and Scottish isolates were assigned to VCG5. According to the banding pattern on AFLP gels VCG2 and VCG5 had the highest frequency compared to the other isolates in NA and Israeli isolates.

Temporal fluctuations in plant parasitic nematode population densities in corn across various Nebraska cropping environments

J. L. BEHN (1), T. Jackson (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 100:S182

Behavioral differences have been observed among genera of plant parasitic nematodes with respect to movement within the soil profile. Nematode migration through the soil complicates recommendations for sampling strategies. It is not clear what environmental or biological conditions determine why and which nematode genera migrate. Samples were collected at monthly intervals, as weather permitted, from 8 locations with varying irrigation practices, cropping history, and nematicide use. All four Fullerton, NE sites showed ectoparasitic nematode genera population densities (*Xiphinema* spp., *Trichodorus* spp., and *Tylenchorynchus* spp.) that increased over the winter months from November 2008 to May 2009 in the absence of a host crop. At one Ewing, NE location, the *Xiphinema* spp. population density trends were similar to Fullerton, increasing over the winter months. However, at a second site in Ewing, the population densities of *Xiphinema* spp. decreased, while at the remaining two sites in Ewing, *Xiphinema* spp. populations held steady over the winter. *Trichodorus* spp. were found in only locations 3 and 4 at the Ewing site, and the population densities decreased over the winter for both locations, contradictory to *Trichodorus* spp. at the Fullerton site. It is difficult to interpret the reasons for differences in these population density trends since these preliminary data are inconclusive. Continued sampling of the sites is planned over the next calendar year to identify the trends of the nematode genera so that sampling recommendations can be improved for nematodes of corn.

The effect of foliar fungicide timing on yield and grain fill in high and low aphid pressure environments

N. R. BESTOR (2), D. S. Mueller (2), A. E. Robertson (2), R. Ritson (2), M. O'Neal (1)
(1) Department of Entomology, Iowa State University; (2) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 100:S182

With the arrival of two invasive pests of soybean, the soybean aphid and soybean rust, there is increasing interest in the use of pesticides for soybean production. Recently, application of foliar fungicides, and to some extent foliar insecticides, to soybean to increase overall "plant health" has been promoted. But the economic benefits of such applications are inconsistent and not well documented. In 2008, the effect of three foliar fungicides (a strobilurin, a triazole and a premix of strobilurin and triazole) applied at growth stages R1 or R3 on seed size and yield was evaluated at two locations in Iowa, one in southeast and one in northwest. Foliar fungicides applied to soybeans in northwest Iowa had a significant positive effect on yield and seed size, while fungicides applied to soybeans in southeast Iowa did not affect yield or seed size. These results were not expected since higher foliar disease levels occurred in southwest Iowa. At both locations, an application of fungicide at R3 resulted in significantly greater yields than an application at R1. In northwest Iowa, no differences in yield were observed between fungicides; however, seed size at this location was significantly greater when a fungicide containing a strobilurin was used. Soybean aphid pressure in northwest Iowa was very high (cumulative aphid days [CAD] = 92,281) while in southeast Iowa, aphid pressure was over 100 fold lower (CAD = 695), which may have been a confounding factor. We plan to investigate the effect of foliar fungicide applications in combination with foliar insecticides under different environmental conditions in subsequent years.

Genetic diversity of *Cercospora sojina* revealed by amplified fragment length polymorphism markers

C. A. BRADLEY (2), A. Wood (3), G. Zhang (2), J. Murray (3), D. Phillips (1), R. Ming (3)
(1) University of Georgia, Department of Plant Pathology, Griffin, GA, USA; (2) University of Illinois, Department of Crop Sciences, Urbana, IL, USA; (3) University of Illinois, Department of Plant Biology, Urbana, IL, USA
Phytopathology 100:S182

Cercospora sojina, a phytopathogenic fungus, causes frogeye leaf spot (FLS) of soybean. Losses caused by this disease in the United States were estimated to range from 6.9 million to 12.7 million bushels annually from 2004 to 2007. The genetic diversity of *C. sojina* isolates collected from three countries was

estimated using amplified fragment length polymorphism (AFLP) markers. A total of 64 isolates of *C. sojina* were analyzed by eight AFLP primer combinations, generating 40 markers. The average genetic similarity of the 64 isolates was 0.56 on a scale between 0 and 1, indicating a high degree of genetic diversity within the species. Cluster analysis resulted in two major clusters and seven sub-clusters. Two isolates collected from Georgia were the most closely related, sharing a genetic similarity of 0.97. Two isolates from China were clustered together. Besides these four samples, no clear separation of isolates based on origin was found. This suggests that genetic diversity within a population is as great as between populations based on locations. Our results provide evidence that substantial genetic diversity exists within the species *C. sojina* and that selection for broad spectrum host-resistance should be targeted in soybean breeding programs.

Progress towards generation of plant ant-FvTox1 antibodies against a *Fusarium virguliforme* toxin that induces sudden death syndrome in soybean

H. K. BRAR (1), M. K. Bhattacharyya (1)
(1) Dept. of Agronomy and Interdepartmental Genetics Program, Iowa State University, Ames, IA, USA
Phytopathology 100:S183

Sudden death syndrome (SDS), caused by *Fusarium virguliforme (Fv),* is a serious soybean disease. It is hypothesized that the foliar SDS symptoms are caused by a phytotoxin(s), released to the roots by the fungal pathogen. A low molecular weight protein (~13.5 kDa) has been shown to produce foliar SDS. The proteinaceous toxin was named as FvTox1. Mice monoclonal antibodies were generated against FvTox1. We have cloned two single chain variable fragment (scFv) antibodies from the hybridoma cell lines that express the mice monoclonal anti-FvTox1 antibodies. Through western blot analysis, we have shown that the recombinant scFv's anti-FvTox1 proteins expressed in *Escherichia coli* can bind to the *E. coli* expressed recombinant FvTox1 protein. Two recombinant scFv's anti-FvTox1 genes are currently being expressed in transformed soybean calli. We will investigate if any of the scFv's anti-FvTox1 proteins (plant anti-FvTox1 antibodies) expressed in transformed soybean calli can bind to the FvTox1 protein. If we can show successful expression of the plant anti-FvTox1 antibodies in transformed calli, stable transgenic soybean lines will be created to stably express the plant anti-FvTox1 antibodies. The transgenic lines expressing detectible levels of the plant antiFvTox1 antibodies will be then investigated to determine if the plant anti-FVTox1 antibodies can suppress the development of foliar SDS.

Corn ear insect damage, fungal infection severity, and mycotoxin concentrations across varying Bt resistance platforms in Nebraska corn fields with natural insect infestation

K. BRAUER (2), R. Wright (1), T. Jackson (2)
(1) University of Nebraska Lincoln, Dept of Entomology; (2) University of Nebraska Lincoln, Dept of Plant Pathology
Phytopathology 100:S183

Ear rotting fungi are common in field corn. While they may not drastically reduce yield directly, secondarily they can contaminate grain with mycotoxins, for which producers can be severely penalized. Ear feeding insects play an important role in fungal colonization of the corn ear by creating wounds that serve as infection points. Insects can be managed with the use of *Bacillus thuringiensis* (Bt) proteins in corn. Field trials with two planting dates were established at two locations in 2007 and 2008 to test this hypothesis under natural insect infestation that included western bean cutworm, corn ear worm, and European corn borer. Treatments consisted of similar corn hybrids with genes for cry proteins Cry1F and Cry1Ab, stacked with cry proteins for rootworm resistance, and their near isogenic line counterparts. At crop maturity, ears were manually harvested and the severity of insect injury and visible fungal infection was determined. Fungal infection rates were recorded from kernels cultured on PDA and fumonsin levels were analyzed with a competitive direct ELISA test. Insect damage severity in 2007 was minimal, but was greater in all later planting dates in both years. Variance between treatments was not significant, but positive correlations between insect damage, Fusarium ear rot and kernel infection, and fumonisin concentration were identified. When analyzed in classes, Bt hybrids, stacked Bt hybrids, and isogenic lines; Bt hybrids and stacked Bt hybrids provided significant ($P \leq 0.01$) reductions in the severity of insect damage, ear rot diseases, fungal kernel infection, and fumonisin concentration. Higher levels of insect damage were observed in 2008 than the previous year, but insect pressure was likely still not severe enough to detect a difference between cry protein treatments.

Identifying pre-plant risk factors for *Bean pod mottle virus* in Iowa

E. BYAMUKAMA (1), A. Robertson (1), F. Nutter (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 100:S183

Integrated disease management requires a thorough understanding of pathogen-plant-environment interactions in order to develop cost-effective management programs. Knowing pre-plant risk factors associated with *Bean pod mottle virus* (BPMV) would enable soybean producers to deploy management practices that delay early season BPMV infection and spread to minimize negative impacts on soybean yield and quality. Potential abiotic and biotic BPMV risk factors identified by correlation analysis were evaluated using regression analysis to quantify the predictive power of single and combined factors at the county scale. We examined thirteen factors: county centroid latitude, longtitude, and elevation; soybean planting date, number of soybean farms, and soybean acres; number of alfalfa acres harvested; for the period of October through April, number of days with daily mean temperature < 0°C, number of days with snow cover, consecutive days with maximum temperature < 0°C, consecutive days with snow cover, and accumulated snow depth; and for March, number of days with mean temperature below 0°C. Variables with highest predictive value for BPMV incidence were days with mean temperatures < 0°C in March and number of soybean farms within Iowa counties, with partial coefficients –4.03 (X_1), and –0.012 (X_2), respectively. The multiple regression model explained 54.5% of the variation in county-scale BPMV incidence; higher BPMV incidence was associated with days in March with mean temperatures < 0°C (X_1) and fewer soybean farms per county (X_2). Thus, we suggest that using the March temperature data and the number of soybean farms/county, potential BPMV incidence can be predicted before planting. Pre-plant predictions can aid soybean growers and seed companies in making management decisions, such as the need for seed and/or foliar insecticide treatments, and selection of planting sites with reduced risk.

The inheritance of mefenoxam resistance in single-zoospore isolates of *Phytophthora erythroseptica*

V. CHAPARA (1), R. J. Taylor (1), J. S. Pasche (1), N. C. Gudmestad (1)
(1) Department of Plant Pathology, North Dakota State University, Fargo, ND, USA
Phytopathology 100:S183

Pink rot of potato, caused by a homothallic diploid Oomycete *Phytophthora erythroseptica*, is reported to be an economically important disease in the United States and known to vary markedly in its sensitivity to the phenylamide fungicide mefenoxam. Previous studies using single-oospore populations of *P. erythroseptica* suggested that mefenoxam resistance was inherited quantitatively. A study was conducted with eight hundred single-zoospore isolates of *P. erythroseptica*, produced from the eight parental isolates having varying sensitivity (2 resistant, 4 intermediately resistant and 2 sensitive isolates) to mefenoxam. In vitro assays were conducted with mefenoxam concentrations of 0, 0.01, 0.1, 1.0, 10.0 and 100.0 µg/ml for isolates with sensitive and intermediate fungicide responses, for resistant isolates higher concentrations of 0, 1, 10, 100, 200 and 300 µg/ml were used. In all instances each isolate was tested twice. The progeny of sensitive (EC50 < 1 µg/ml) isolates had the same phenotype as the parents, with no major shift towards increased insensitivity to the fungicide. Similarly, the progeny from resistant parents (EC50 > 100 µg/ml) were also resistant to mefenoxam, however, the progeny from one parent were less insensitive to the fungicide and the progeny from the other parent were generally more insensitive. All of the single-zoospore progeny derived from the four intermediately resistant isolates (EC50 values range from 1 to 99 µg/ml) had the same phenotype as the parental isolates with progeny of two parents trending towards increased insensitivity while the progeny of another parent generally had decreased insensitivity to the fungicide. These results on the inheritance of mefenoxam resistance using single-zoospore progeny of *P. erythroseptica* do not support the conclusions of previous studies that mefenoxam resistance is inherited quantitatively.

A survey of *Venturia inaequalis* fungicide resistance in Indiana and Michigan apple orchards

K. S. CHAPMAN (1), K. L. Quello (1), J. L. Beckerman (1)
(1) Purdue University, West Lafayette, IN, USA
Phytopathology 100:S183

Apple growers rely heavily on fungicides to manage *Venturia inaequalis*, the fungus that causes apple scab. Fungicide resistance has developed as a result. To quantify and assess the levels of fungicide resistance, isolates of *V. inaequalis* were collected from Indiana and Michigan orchards and fungicide resistance was evaluated. Previously published works were used to determine the baseline concentrations of fungicides and thresholds for growth. Differences were found in the levels of resistance between the two states. In Michigan, 2.0% of the isolates tested were resistant to Sovran (defined as 90% relative growth in the presence of fungicide), but 52.9% were shifted and less sensitive to the fungicide. 63.5% of MI isolates were resistant to Topsin M. With respect to Dodine, 13.5% were resistant (90% relative growth), but 67.3% showed a shift in resistance. 42.3% of isolates tested with Nova were resistant (80% relative growth), and resistance had shifted in 55.8% of isolates. For Indiana, there was no indication of resistance to Sovran. 86.6% of

isolates tested had resistance to Topsin M. Dodine testing showed that 7.3% of isolates were resistant and 62.2% had shifted resistance. Of IN isolates tested with Nova, 34.1% were resistant and 57.3% were shifted in their resistance. On a state level this survey will provide the opportunity to educate growers on the degree of fungicide resistance present in local orchards and prevent ineffective fungicide applications.

Transient expression of MFSV genes in Drosophila S2 cells

F. M. CISNEROS (4), C. Tsai (3), A. E. Whitfield (2), S. A. Hogenhout (1), M. G. Redinbaugh (5)
(1) John Innes Centre, UK; (2) Kansas State University; (3) National Taiwan University, Taiwan; (4) The Ohio State University; (5) USDA-The Ohio State University
Phytopathology 100:S184

Maize fine streak virus (MFSV) is a member of the genus Nucleorhabdovirus that is transmitted by the leafhopper *Graminella nigrifons*. The virus replicates in both its maize host and its insect vector. To determine whether Drosophila S2 cells support the production of full-length MFSV proteins, we inserted the open reading frames for the nucleoprotein (N), phosphoprotein (P) and replicase protein (L) of MFSV into the pMT/V5-His-Topo vector to produce V5 epitope/ 6X His tagged proteins. The S2 cells were transfected with these plasmid constructs. When analyzed by western blot, antibodies to the V5 epitope clearly reacted with proteins of ~55 and 43 kDa in cells transfected with plasmids carrying the N and P genes, respectively, the sizes expected for the full-length fusion proteins. No bands were detected in non-transfected Drosophila S2 cells. The expression of the N gene was also tested with antibodies raised against MFSV virions, which detects the N protein as well as several other viral proteins. MFSV virion antibodies detected a protein of ~55 kDa in S2 cell protein extracts. Antibodies raised against a peptide sequence from the deduced MFSV P protein reacted with a protein of ~ 43 kDa in transfected S2 cell protein extracts. The expression of the MFSV N and P genes were detected over a period of 4 days after induction of gene expression with $CuSO_4$, but were not detected in cells not exposed to $CuSO_4$. Experiments are underway to asses MFSV L gene expression in S2 cells. Our results indicate that Drosophila S2 cells can steadily express full-length N and P proteins for at least 4 days. This finding is important in order to optimize Drosophila S2 cell system conditions for construction of an infectious full-length clone of MFSV.

***Pantoea stewartii* subsp. *stewartii* carries two type III secretion systems required for adaptation to insect vector and plant hosts**

V. R. CORREA (3), D. R. Majerczak (5), E. Ammar (2), M. Merighi (5), C. Exner (3), D. L. Coplin (5), R. C. Pratt (3), M. G. Redinbaugh (1), S. A. Hogenhout (4)
(1) ARS, USDA Corn and Soybean Research, Plant Pathology, OARDC/The Ohio State University; (2) Entomology, OARDC/The Ohio State University; (3) Horticulture and Crop Science, OARDC/The Ohio State University; (4) John Innes Centre, Norwich, UK.; (5) Plant Pathology, The Ohio State University
Phytopathology 100:S184

Animal and plant pathogenic bacteria interact with their hosts by injecting virulence proteins into host cells via type III secretion systems (TTSS). The Hrc-Hrp cluster of *Pantoea stewartii* subsp. *stewartii* (Pnss), the causative agent of Stewart's wilt in maize (*Zea mays* L.), was previously shown to be important for pathogenicity in plants. Pnss has a second TTSS (PSI-2), that is similar to the invasion-associated TTSS, typical of animal pathogens. We hypothesized that PSI-2 is required for Pnss colonization of its vector, the maize flea beetle, Chaetocnema pulicaria. The PSI-2's psaN gene, which encodes an ATPase essential for building the injectisome and secretion of effectors, was inactivated with transposon insertions and frame-shift mutations. Beetles were allowed to feed on plants infected with Pnss mutants or wild-type bacteria. Insect colonization by Pnss mutants and wild type bacteria was analyzed using immunofluorescence confocal microscopy of dissected insect organs or using viable cell counts of insect homogenates. Pnss carrying transposon insertions and frame-shift mutations negated bacterial persistence in flea beetle guts and reduced subsequent transmission to maize. Complementation of mutants with plasmids carrying the psaN+ gene partially restored bacterial persistence and transmission. Pnss psaN mutants were fully virulent on maize, indicating that PSI-2 was not required for plant pathogenicity. Our results demonstrate that the multiple TTSS in Pnss are functionally active and play different roles in adaptation of the bacterium to insect and plant hosts.

Interactions between lesion nematodes and fungal pathogens on maize seedlings

M. P. DA SILVA (1), G. P. Munkvold (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 100:S184

Lesion nematodes (*Pratylenchus penetrans*), are well known to have interactions with root rot pathogens on a wide variety of host plants. The objective of this research was measure the effects of *P. penetrans* infestation on seedling disease symptoms caused by fungal pathogens (Rhizoctonia and Fusarium spp.), assess the impact of nematode control with abamectin on above pathogens and evaluate potential added seedling disease management benefit of abamectin combined with commercial fungicide seed treatment on maize. In a greenhouse experiment, 150 ml pots filled with autoclaved sand-soil mixture with a layer of fungal inoculum (colonized corn meal/sand mixture) on top of the seed. A suspension of 1000 *P. penetrans* (adults, juveniles and eggs) was added to the pots at the time of planting. A factorial experimental design was used including 8 seed treatments × 4 pathogen treatments × 4 reps. Experiments were harvested 30 days after planting. Emergence was evaluated at 8, 13 and 20 days after planting. Shoot lengths, fresh and dry shoot weights, fresh and dry root weights and root health were determined. Roots were scanned and image analysis conducted with WinRhizo software; root length, root volume and root branching were determined. The results suggest significant effects on root health with interactions between fungal pathogens/root-lesion nematodes and between seed treatment/fungal inoculation. Results also suggest significant effects on root length and root branching for fungal inoculation. *R. solani* had a greater effect on emergence than *F. verticillioides*. Further root health analysis will be conducted with WinRhizo.

Development of a forecasting model to estimate risk of Sclerotinia stem rot development on canola in North Dakota

L. E. DEL RIO (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 100:S184

Sclerotinia stem rot (SSR) is an important yield reducing disease that is endemic to canola producing areas of North Dakota. SSR, which is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is managed mainly through the use of fungicides. Weather conditions play an important role in development of SSR epidemics and thus on the profitability of fungicide applications made to control it. A warning system aimed at estimating the risk of development of SSR epidemics was produced using logistic regression analysis, disease data collected from more than 800 fields through field surveys and weather data collected through a net of 27 weather stations. The selected model had a *c* value of 0.79 and a Somers' D value of 0.58, and identified rain and solar radiation as independent variables of importance. When validated using a data set of similar size that had not been used in model development, the model produced a true positive fraction of 64% and a true negative fraction of 74% and an overall accuracy of 72%. The model was available to canola growers through a website in 2008.

Assessment of soybean genotypes for resistance to *Pythium* spp.: Key to managing this seedling disease complex

M. L. ELLIS (1), P. A. Paul (1), A. E. Dorrance (1)
(1) Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH, USA
Phytopathology 100:S184

Resistance to *Pythium* spp. is not well known in soybean cultivars, especially for those species most prevalent in Ohio soybean fields. The objective of this research was to begin screening for resistance to *P. irregulare* and *P. ultimum* var. *sporangiiferum*. A greenhouse assay was used to evaluate 96 soybean lines for potential resistance to two isolates of *P. irregulare*, followed by an evaluation of the top performing lines with two isolates of *P. ultimum* var. *sporangiiferum*. For both assays, data for seed germination, total weight, root weight, and a root rot score using an ordinal scale were collected. Based on the results from the two assays, there were no significant interactions between isolates within species and lines. There was a significant difference between the two isolates of *P. irregulare* and among lines for the initial screening. Thirty two lines were screened with *P. ultimum* var. *sporangiiferum* and there was a significant difference between isolates for root weight. PI 424354 had the highest weight following inoculation with *P. irregulare*; however, it performed poorly, compared to the other lines, when inoculated with *P. ultimum* var. *sporangiiferum*. Of the 32 lines screened, none were resistant to one of the *P. ultimum* var. *sporangiiferum* isolates. These results suggest that there is potential resistance to both *Pythium* spp.; however, this resistance may not confer resistance to all isolates within and across species.

De-acclimation and re-acclimation responses to sudden temperature shifts in *Lolium perenne*

J. D. FARRELL (1), U. Frei (1), S. Fei (1), T. Lubberstedt (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 100:S184

Climate change has resulted in a higher variability in climate patterns; exposing plants to frequent freeze thaw cycles especially during the late winter and early spring. Perennial ryegrass (*Lolium perenne*) was chosen as model for investigating cold acclimation and freezing tolerance in relation to shifting temperatures. Perennial ryegrass is an important crop in Europe, Asia and Africa as both forage and turf grass. In the United States perennial ryegrass has the potential to become a cover crop in maize fields where stover is removed. Recently, genomic resources have become available including ESTs, microarrays and BAC libraries. Preliminary frost tolerance assays, also known as ion leakage assay have revealed an interesting pattern between two Mediterranean cultivars. One cultivar acclimated quickly however as the cold temperatures continued the frost tolerance decreased compared to the other cultivar, which acclimated slowly and was able to sustain frost tolerance. The objective of this study is to determine the frost tolerance of these two Mediterranean cultivars during cold acclimation, de-acclimation and re-acclimation, simulating the typical pattern of a late winter thaw cycle. In parallel mRNAs will be collected from each cultivar during normal, cold acclimation, de-acclimated and re-acclimation conditions for cDNA microarrays assays. Comparing gene expression between the two Mediterranean cultivars during different temperature conditions will help identify molecular mechanisms involved in acclimation, de-acclimation and re-acclimation. The long term goal of this project is to identify the candidate genes involved in these acclimation processes, to find the genomic location of these genes and to extract the full length gene and promoter sequence; in the hopes of expanding our knowledge to other crop species.

Low lignin (brown midrib) sorghum genotypes restrict growth of Fusarium spp. as compared with near-isogenic wild-type sorghum

D. L. FUNNELL-HARRIS (1), J. F. Pedersen (1), S. E. Sattler (1)
(1) USDA-ARS, Grain, Forage and Bioenergy Research, University of Nebraska, Lincoln, NE, USA
Phytopathology 100:S185

To increase usability of sorghum for bioenergy and forages, two different brown midrib (*bmr*) genes, *bmr*-6 and *bmr*-12, were backcrossed into five elite backgrounds, resulting in reduced lignin near-isogenic genotypes. Field-grown grain from *bmr*-6 and *bmr*-12 plants had significantly reduced colonization by *Fusarium moniliforme sensu lato* as compared with wild-type grain. *Fusarium* isolates were identified to species using sequence analysis of the translation elongation factor gene. Three of the most commonly identified species, *Fusarium thapsinum, Fusarium proliferatum* and *Fusarium verticillioides*, were members of *F. moniliforme* and included sorghum pathogens. Three other commonly isolated species, *Fusarium bullatum, Fusarium pallidoroseum* and *Fusarium graminearum*, likely colonize sorghum asymptomatically. Chi-square analyses showed that the ratios of *Fusarium* species colonizing *bmr*-12 grain were significantly different from those of wild-type, indicating that *bmr*-12 affects colonization by *Fusarium* spp. across genetic backgrounds. A thrice-replicated bioassay was conducted in which peduncles of wild-type and near-isogenic *bmr* genotypes in a single background were inoculated with fungi associated with sorghum. *F. thapsinum, F. verticillioides, Fusarium armeniacum* and *Alternaria alternata* were pathogenic on wild-type plants in most cases. Lesion lengths were significantly reduced on one or both *bmr* genotypes infected by *F. verticillioides, F. thapsinum* or *A. alternata* compared to lesions produced on near-isogenic wild-type plants. These data indicate that *bmr*-6 and *bmr*-12 affect colonization by *Fusarium* spp. and *A. alternata*.

Evaluation of aggressiveness and host range of *Fusarium acuminatum* and *Fusarium redolens* associated with root rot of dry beans

A. GAMBHIR (1), R. S. Lamppa (1), J. B. Rasmussen (1), R. S. Goswami (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 100:S185

Dry bean (*Phaseolus vulgaris*), a favored rotational crop with high nitrogen fixing ability and food value is affected by a large number of fungal diseases. Production of this crop in the US is primarily concentrated in the North Central region of the country, where Fusarium root rots are a major concern. *Fusarium solani* f. sp. *phaseoli* has been considered as the primary causal agent of this disease. However, our findings suggest the involvement of other *Fusarium* species. Among these, *Fusarium acuminatum* and *Fusarium redolens*, were detected for the first time in 2007 in North Dakota and Minnesota, on roots of dry bean plants collected from root rot afflicted fields. Roots of the infected plants exhibited reddish brown lesions or discoloration on hypocotyl and tap roots, characteristic of Fusarium root rot in dry beans. Koch's postulates were completed for these species. Variation in aggressiveness on dry beans among isolates and their ability to infect crops commonly grown in rotation with dry beans was evaluated in greenhouse trials. Isolates of *F. acuminatum* and *F. redolens* from dry beans exhibited pronounced differences in the ability to cause disease on a highly susceptible kidney bean cultivar. Some of the isolates evaluated were as aggressive as *F. solani* f. sp. *phaseoli*. Aggressive isolates from both species were able to infect barley, canola, chickpeas, corn, field pea, flax, lentils, potato, soybeans, sugarbeet, sunflower and wheat. But the disease severity and symptoms developed varied between hosts. These findings suggest a possible change in *Fusarium* species causing root rots of dry beans in this region and highlight the potential threat posed by them to production of dry beans and other crops grown in rotation.

Improving management of soybean cyst nematode through extension demonstration and outreach

L. J. GIESLER (13), C. Bradley (10), A. Dorrance (8), T. Niblack (9), G. Tylka (1), D. Jardine (2), D. Malvick (11), L. Sweets (12), S. Markell (4), L. Osborne (7), P. Esker (14), G. Bird (3), J. Faghihi (6), A. Tenuta (5)
(1) Iowa State University; (2) Kansas State University; (3) Michigan State University; (4) North Dakota State University; (5) Ontario Ministry of Agriculture, Food & Rural Affairs; (6) Purdue University; (7) South Dakota State University; (8) The Ohio State University; (9) USDA/ARS/University of Illinois; (10) University of Illinois; (11) University of Minnesota; (12) University of Missouri; (13) University of Nebraska-Lincoln; (14) University of Wisconsin
Phytopathology 100:S185

While soybean cyst nematode (SCN) is the most yield limiting pest of soybean in the United States, soybean growers are not always properly managing it. Recent surveys have demonstrated this in Iowa and direct correspondence with growers and commercial agriculture professionals quickly reveals that a major problem exists in that this pest is often ignored. Extension plant pathologists and nematologists from the North Central states are collaborating in this project to deliver a consistent message on management of SCN. As a part of the project, a total of 28 replicated on-farm strip trials were established to evaluate the influence of SCN resistance source on yield and SCN reproduction across the North Central states. Soybean yields were measured, and SCN populations were determined in the spring and fall for all locations. In addition, each location was tested for SCN population HG type, which identifies the ability of the population to reproduce on each of the resistance sources used in the trials. Yield was consistently higher in resistant cultivars compared to susceptible varieties, but response of cultivar varied with location. The yields were highest for varieties utilizing the Peking source of resistance, which had a 5.3 bu/A yield advantage over susceptible varieties averaged over all locations. In fields with high SCN populations ($\geq$3,000 eggs/100 cc soil), the average yield advantages of varieties utilizing the Peking, PI 88788, and Hartwig sources of resistance were 15.5, 11.8, and 6.3 bu/A better than the susceptible varieties, respectively. In addition to research plots, team members developed extension programs on SCN and delivered SCN information in all states. A total of 30 field days and 33 indoor education programs were delivered to over 5,000 participants in 50 hours of programming.

Recovery of *Phakopsora pachyrhizi* urediniospores from passive spore trap slides and extraction of their DNA for quantitative PCR

J. S. HAUDENSHIELD (2), P. Chaudhary (2), G. L. Hartman (1)
(1) USDA-Agricultural Research Service, Urbana, IL, USA; (2) University of Illinois, Urbana, IL, USA
Phytopathology 100:S185

Enumeration of rust spores from passive spore traps utilizing white petrolatum-coated slides by traditional microscopic evaluation can represent a serious challenge. Many fungal spores look alike, and clear visualization on the adhesive can be obscured by particulate debris or nonuniformities within the adhesive layer; reports will commonly describe only the number of "rust-like" spores. Molecular methods of *P. pachyrhizi* detection, utilizing both standard PCR and quantitative PCR (qPCR), have been available for several years, but extraction of fungal DNA from petrolatum-embedded spores remained difficult. We now demonstrate the utility of a novel method for recovering the petrolatum layer carrying trapped spores from slides using biodegradable foam strips, with subsequent DNA extraction to yield material suitable for quantification by qPCR. This method permits even single spores of *P. pachyrhizi* to be recovered and detected. False-negative calls were minimized by using a multiplexed exogenous control; no false-positives were observed. This method was successfully employed to assess spore loads in passive traps located at sentinel plots in the USA during the 2008 soybean growing season.

Evaluation of extraction methods for detecting *Xanthomonas axonopodis* pv. *phaseoli* in common bean seed

Y. HE (1), G. Munkvold (1)
(1) Seed Science Center, Iowa State University, Ames, IA, USA
Phytopathology 100:S185

Xanthomonas axonopodis pv. *phaseoli* (Xap) and Xap var. fuscans are important seedborne pathogens of *Phaseolus vulgaris*. In order to maintain seed quality and meet phytosanitary requirements, accurate seed health testing methods are critical. Currently accepted methods for these pathogens include several variations on extraction methods; therefore our objective is to assess

the influence of different extraction steps on the sensitivity of Xap detection in *P. vulgaris* seeds. Seeds were inoculated with Xap to reach inoculum levels from 101 CFU/seed to 105 CFU/seed and mixed with clean and healthy *P. vulgaris* seeds. One contaminated seed was mixed into each 1000-seed subsample. Thirty 1000-seed subsamples were tested for each different extraction condition. Extraction methods tested included soaking whole seeds in sterilized saline phosphate buffer overnight at 4°C and at room temperate for 3h, soaking with and without vacuum, and concentrating the seed extract by centrifuging. The seed extract dilutions were cultured on semi selective agar media MT and XCP1. The proportions of positive subsamples were recorded and compared to measure the effects of each extraction step on detection sensitivity. The results showed that vacuum extraction and centrifugation of seed extracts increased sensitivity, and soaking overnight at 4°C was more effective than soaking at room temperature for 3h. Our results suggest that a centrifugation step would be a valuable addition to the current method approved by the International Seed Testing Association (ISTA), but these results should be confirmed using naturally infected seedlots.

Correlation between Fusarium head blight severity and deoxynivalenol in three winter wheat cultivars

J. HERNANDEZ NOPSA (1), S. Wegulo (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 100:S186

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a damaging disease of wheat. In 2008, a field experiment was conducted to identify relationships between visual assessments of FHB and deoxynivalenol (DON) in three winter wheat cultivars. The cultivars Jagalene, Harry, and 2137 were planted following corn on 27 October 2007. In May 2008, plots were inoculated with 1 × 105 spores/ml of *F. graminearum* at early anthesis and were not irrigated. There also was heavy natural inoculum. Cultivars were arranged in a randomized complete block design with three replications. FHB severity was determined 21 days after inoculation on 20 heads tagged in each of 13 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 70, and 90%. There was a significant positive correlation between FHB severity and DON in all three cultivars: Jagalene (r = 0.92, P < .0001); Harry (r = 0.64, P = 0.0176); and 2137 (r = 0.88, P < 0.0001). DON concentration was lower (P = 0.05) in 2137 than in Harry or Jagalene; it was highest in Harry (32 µg/g) followed by Jagalene (29 µg/g) and 2137 (19 µg/g). This study demonstrated (i) a positive correlation between FHB severity and DON and (ii) differences among cultivars in the levels of DON they accumulated. Similar results were obtained in 2007.

Evaluation of winter wheat cultivars for resistance to Fusarium head blight and deoxynivalenol

J. HERNANDEZ NOPSA (1), S. Wegulo (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 100:S186

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, can cause significant losses. The reaction to FHB and deoxynivalenol (DON) of the winter wheat cultivars Jagalene, Harry, 2137, Hondo, Alliance, Infinity, Goodstreak, Karl 92, Wahoo, Millennium, Wesley, and Overley was evaluated in the field in 2008. In addition to natural inoculum, plots were inoculated with 1 × 105 spores/ml of *F. graminearum* at early anthesis and were not irrigated. FHB index, the percentage of *Fusarium*-damaged kernels (FDK), DON, yield, 1000 kernel weight (1000kwt), and test weight (twt) were measured. Differences among cultivars were significant ($P \leq 0.0068$) for FHB index, FDK, DON, 1000kwt, and yield. Ranges of measured variables were: FHB index: 13% (Harry) to 64% (Overley); FDK: 21% (2137) to 42% (Harry and Wahoo); DON: 3.7 µg/g (Karl 92) to 9.9 µg/g (Harry); yield: 763 kg/ha (Wahoo) to 1,365 kg/ha (Karl 92); 1000 kwt: 24.8 g (Wahoo) to 30.8 g (2137). FDK and DON were positively correlated (r = 0.59, P = 0.0442). There was a significant (($P \leq 0.05$) negative correlation between FDK and yield ((r = –0.74), FDK and twt ((r = –0.64), FDK and 1000 kwt ((r = –0.84), FHB index and twt ((r = –0.69), and DON and twt ((r = –0.74). This study demonstrated differences among winter wheat cultivars in their reaction to FHB and DON. Interestingly, Harry had the lowest FHB index but the highest DON level, implying that cultivars with resistance to FHB may be susceptible to DON accumulation.

Spatial and temporal analysis to find the epicenters of soybean rust disease foci using remote sensing, GPS and GIS technologies

N. S. HOLAH (1), D. F. Narvaez (2), J. J. Marois (3), D. L. Wright (3), F. W. Nutter (1)
(1) Iowa State University, Plant Pathology Department, Ames, IA, USA; (2) Monsanto Co, St. Louis, MO; (3) North Florida Research and Education Center, University of Florida, Quincy, FL
Phytopathology 100:S186

Exotic plant pathogens have the potential to dramatically impact the U.S. agricultural economy. New plant pathogen threats may be deliberately or accidentally introduced, or may be introduced by a natural event (e.g., hurricanes). In order to minimize injury to susceptible crops, a precise and accurate early warning system is needed to detect, correctly identify, and quickly respond to new plant pathogen threats. The integration of Global Positioning Systems (GPS), Geographic Information Systems (GIS), and remote sensing technologies offer tremendous opportunities for meeting U.S. agricultural biosecurity needs. The objective of this study was to detect the focal epicenters of Asian soybean rust where this plant pathogen was deliberately introduced to soybean field plots. Pathogen-specific temporal and spatial signatures were extracted from high-resolution satellite images of soybean plots inoculated with Asian soybean rust in Quincy, FL. Disease foci epicenters were determined using high resolution satellite imagery obtained on 27 August, 21 September, and 29 September 2006. Image intensities were extracted from plot images for each date. Contour maps and kriging were used to map and identify the GPS locations of soybean rust disease foci and foci epicenters. The use of integrated GPS, GIS, and remote sensing technologies accurately determined the GPS coordinates where the pathogen was deliberately introduced into plots. The GPS coordinates of the predicted locations of epicenters differed only 1.5 ± 0.92 m from stated inoculation points.

A cyst nematode effector protein appears to modulate numerous plant molecular processes

P. J. Howe (1), T. Hewezi (1), T. R. Maier (1), R. S. Hussey (2), E. L. Davis (3), M. G. Mitchum (4), T. J. Baum (1)
(1) Iowa State University, Ames, IA; (2) University of Georgia, Athens, GA; (3) North Carolina State University, Raleigh, NC; (4) University of Missouri, Columbia, MO
Phytopathology 100:S186

Cyst nematodes are some of the most destructive plant pathogens. These biotrophic parasites form elaborate feeding sites, syncytia, in the roots of their host plants and remove valuable nutrients from the plant. During the formation of the syncytium the nematode secretes effector proteins into root cells, which causes extensive molecular changes in the cell and allows the parasite to manipulate cellular processes. In this study we have worked to characterize the sugar beet cyst nematode effector protein 4D09 with the goal to investigate its role in parasitism. For this purpose we assessed the timing of 4D09 gene expression and transferred this gene in the host plant Arabidopsis to assess phenotypic plant changes that could reveal this effector's functions. Furthermore, when using a Yeast Two-Hybrid approach we found that 4D09 interacts with a plant protein that has been shown to be involved in a multitude of molecular processes. By targeting this key molecular regulator protein the nematode might be gaining control of some of the plant cellular processes.

Aggressiveness of isolates of *Phialophora gregata* genotype B from resistant and susceptible soybean monocultures

T. J. HUGHES (1), C. R. Grau (1)
(1) University of Wisconsin, Department of Plant Pathology, Madison, WI, USA
Phytopathology 100:S186

Many soybean accessions described as resistant to brown stem rot (BSR) are preferentially colonized by isolates of *Phialophora gregata* genotype B (*Pg* B). These isolates are generally considered less aggressive than isolates of *Pg* genotype A because they cause mild or no foliar symptoms characteristic of BSR. However, variation in aggressiveness has been observed among isolates of *Pg* B. Monocultures of BSR-resistant or susceptible soybean accessions were planted from 2000 through 2005 to determine if soybean accessions influence the aggressiveness of isolates of *Pg* B. BSR-susceptible Corsoy 79 and BSR-resistant PI 567.157A were inoculated under greenhouse conditions with a total of 39 isolates of *Pg* B obtained from the different monocultures. BSR severity was determined as the percentage of symptomatic foliar and internal stem tissue. Overall, BSR severity was low and did not exceed 20%. Isolates of *Pg* B caused more severe foliar (P < 0.0001) and stem (P = 0.0008) symptoms on PI 567.157A than Corsoy 79. Analysis of stem symptom severity indicated an interaction (P = 0.0124) between soybean accession and the origin of isolates of *Pg*. Isolates of *Pg* B obtained from the monoculture of a BSR-susceptible or resistant accession were more aggressive than isolates from a mixed culture of susceptible and resistant cultivars. The relationship between the origin of isolate of *Pg* B and isolate aggressiveness was more apparent for PI 567.157A than for Corsoy 79.

Phytophthora root rot-like symptoms on soybeans containing *Rps* 1k in Wisconsin in 2008

T. J. HUGHES (2), P. D. Esker (2), S. P. Conley (1)
(1) University of Wisconsin, Department of Agronomy, Madison, WI, USA; (2) University of Wisconsin, Department of Plant Pathology, Madison, WI, USA
Phytopathology 100:S186

Cool and wet conditions during the early 2008 growing season in Wisconsin were conducive for diseases like Phytophthora root rot (PRR), caused by

Phytophthora sojae (*Ps*). While conditions had reversed by August and many areas were drought-like, symptoms characteristic of PRR began to appear in several fields. Since many of these fields were planted to cultivars containing *Rps* 1k, serious concern arose over the breakdown of resistance conferred by this gene. To determine if these symptoms were associated with colonization by *Ps*, soybean plants were collected from 22 fields in 7 counties and assayed for the presence of *Ps*. In all plant samples, *Ps* was neither isolated nor observed. Instead, numerous isolates of *Diaporthe phaseolorum* var. *caulivora* (*Dpc*), *D. phaseolorum* var. *sojae* (*Dps*), and *Macrophomina phaseolina* (*Mp*) were obtained. Northern stem canker and pod and stem blight are caused by *Dpc* and *Dps*, respectively, while *Mp* causes charcoal rot. Based on both field observations and plant samples, the PRR-like symptoms observed in Wisconsin in 2008 were thought to be the result of infection by *Dpc*, *Dps,* or *Mp*. However, greenhouse inoculations with these fungi did not produce symptoms similar to those observed in 2008 on two cultivars containing *Rps* 1k. Whether the PRR-like symptoms were the result of infection by a combination of these fungi or if the plants defense response to *Ps* may have increased susceptibility to *Dpc*, *Dps*, or *Mp*, still remain unknown.

Characterization of two *Arabidopsis* bHLH transcription factors that are induced in cyst nematode syncytia

J. JIN (1), T. Hewezi (1), T. Baum (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 100:S187

The soybean cyst nematode (SCN, *Heterodera glycines*) is a biotrophic endoparasite that annually causes an estimated one billion dollar loss to the United States soybean industry. The model system of *Arabidopsis thaliana* and the sugar beet cyst nematode (BCN, *Heterodera schachtii*), a close relative of SCN, has been used broadly to study the compatible interaction between a cyst nematode and a plant. Successful cyst nematode parasitism relies on the formation and maintenance of feeding sites (syncytia) in host roots through processes that are highly regulated by the interaction between the cyst nematode and the host. By using promoter::GUS fusion constructs, we have discovered that two basic Helix-Loop-Helix (bHLH) transcription factor promoters are induced in syncytia at 3 and 7 days after nematode inoculation and that the syncytium appears to be the only location of coexpression for both genes. We also detected that mRNA abundance of both transcription factor genes was up-regulated in Arabidopsis roots following BCN infection, corroborating our promoter data. Overexpressing bHLH genes in Arabidopsis altered root morphology and changed susceptibility to BCN. By using yeast-two-hybrid analyses and bimolecular fluorescent complementation assays, we determined that the two bHLH transcription factors studied here can form a heterodimer. We hypothesize that this heterodimer specifically forms in the developing cyst nematode feeding site and is involved in the reprogramming of root cells into syncytia. Expression analyses are under way to identify target genes regulated by both transcription factors.

Using BPMV and SMV vector systems to explore soybean cyst nematode-plant interactions

P. S. JUVALE (1), A. Eggenberger (1), C. Zhang (1), J. Hill (1), S. Whitham (1), M. Mitchum (2), T. J. Baum (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA; (2) Division of Plant Sciences and Bond Life Sciences Center, University of Missouri, Columbia, MO
Phytopathology 100:S187

Soybean is one of the main sources of oil and an important source of complete protein worldwide. Among the various pathogens that attack soybean, the soybean cyst nematode (SCN) is especially devastating. In spite of sustained research efforts, an elaborate understanding of plant-nematode interaction is still lacking. Since the available methods to generate transgenic soybean plants are time-, labor- and cost-intensive, there is a critical need for adapting innovative approaches for rapid gene expression or gene silencing in soybean roots in a high through-put manner to elucidate nematode-plant interaction. Due to the rapid pace at which virus infection becomes established throughout the plant and the high yield of viral encoded proteins, plant-virus based vectors present promising tools for expressing foreign proteins in soybean. On the other hand, virus-induced gene silencing (VIGS) is an exceptional reverse genetics tool that can be used to generate mutant phenotypes for unknown genes in soybean. We are using a soybean mosaic virus (SMV) vector for expressing previously identified SCN parasitism genes in soybean and a bean pod mottle virus (BPMV) vector for VIGS to elucidate gene functions in nematode resistant soybeans varieties. Since the infection profile of soybean roots by SMV and BPMV is not clearly understood, our primary goal is to study and optimize viral infection of the soybean root system. Currently, we are using reporter genes, GUS and GFP, to study virus movement to the root, optimize conditions to maximize infected root volume and ensure viral particle replication in the nematode feeding site.

Optimizing extraction of *Fusarium virguliforme* DNA from crop residue and conidia

T. M. KOLANDER (1), D. K. Malvick (1), J. E. Kurle (1)
(1) University of Minnesota, St. Paul, MN, USA
Phytopathology 100:S187

Sudden death syndrome (SDS) of soybean (*Glycine max*), caused by *Fusarium virguliforme* (*Fv*), can cause severe yield losses. Crop rotation is not effective for managing SDS. One explanation is that *Fv* may survive and possibly grow on residue from crops rotated with soybean. We tested three methods for extracting Fv DNA from crop residue and *Fv* conidia for use in PCR. Residue of soybean, corn (*Zea mays*), alfalfa (*Medicago sativa*), and wheat (*Triticum aestivum*) was soaked in a suspension of *Fv* conidia. Residue was buried in pasteurized field soil maintained at ~23°C for 3 and 6 weeks. Modifications of the MoBio UltraClean™ Plant (UCP) kit, FastDNA® (FD) kit, and the MoBio PowerSoil™ (PS) kit were used for residue extractions and the latter two kits were used for extractions from 10^4 to 10^7 conidia. Standard PCR (sPCR) and quantitative PCR (qPCR) were completed using *Fv*-specific primers. The sPCR bands from residue were consistently more intense for the FD kit, especially at 3 weeks post-burial. At 3 weeks, mean qPCR Ct values for the FD kit were on average 0.9, 0.6, 2.8, and 6.5 cycles lower than the PS kit for corn, wheat, alfalfa, and soybean, respectively. At 6 weeks, Ct values resulting from the FD kit were lower only for alfalfa and soybean. The Ct values for soybean, resulting from the FD kit, were 6.3 and 7.3 cycles lower than the UCP kit after 3 and 6 weeks, respectively. Using sPCR and qPCR, quantities of *Fv* DNA obtained with the PS kit correlated with the number of conidia. *Fv* DNA from conidia was not detected with qPCR using the FD kit. The FD kit was generally more effective at extracting *Fv* DNA from crop residue, especially soybean and alfalfa, and the PS kit was superior for extracting DNA from conidia.

Determining specificity of commercially available ELISAs for *Clavibacter michiganensis* subspecies

K. A. KORUS (1), A. D. Ziems (1), A. K. Vidaver (1), T. A. Jackson (1)
(1) University of Nebraska, Lincoln, NE, USA
Phytopathology 100:S187

Clavibacter michiganensis (Cm) subsp. *nebraskensis* (Cmn), the bacterium causing Goss's wilt of corn, is currently diagnosed by symptom identification and successful isolation onto CNS selective medium. An ELISA test kit (Agdia®) specific to Cm *michiganensis* (Cmm) reportedly gives a cross-reaction with Cm subspecies. This ELISA would provide a quick and inexpensive method for diagnosis of Cmn. ELISA test kits were provided by Agdia specific to Cmm, Cm *tessellarius* (Cmt), and Cm *sepedonicus* (Cms), respectively. Also, an ELISA test kit (Neogen®) specific to Cmn was included in the study. For each test kit 13 strains of Cmn, 3 Cmm, 5 Cmt, 3 Cms and 1 Cm *insidiosus* (Cmi) were tested for cross-reaction. Cultures were grown on NBY medium for 24 hr, transferred to liquid nutrient broth and agitated for 72 hr, all at 27°C. The CFU/ml was calculated for each isolate and the optimal concentration needed to produce a positive reaction for each strain. Preliminary results conducted at a concentration of 104 CFU/ml from 2 of 4 replications indicate that all 5 subspecies (but not all strains) tested positive on plates coated with antibodies specific to Cmm, Cmn, and Cmt but not on plates coated with antibodies specific to Cms. ELISAs using antibodies specific to Cmm, Cmn and Cmt could be used to give a cross reaction with Cmn. Additional data will be presented on subspecies specificity of Cmm ELISA test strips.

Optimization of inoculation methods with *Fusarium virguliforme* for virus-induced gene silencing studies on soybean sudden death syndrome

L. LEANDRO (1), V. Silva (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA, USA
Phytopathology 100:S187

Plant age is important in soybean sudden death syndrome (SDS) since root infection of mature plants may not be conducive to foliar symptoms due to restricted xylem colonization. In order to conduct virus-induced gene silencing (VIGS) studies, a method is needed that allows SDS symptoms to develop in plants inoculated with *Fusarium virguliforme* two weeks after inoculation with the virus. The objective of this study was to develop an inoculation method for VIGS studies on SDS. Roots of 13-day-old soybean plants were wounded by longitudinally splitting the tap root or by cutting the tap and lateral roots 1.25 inches below the soil line, then replanting into soil infested with conidia. To test the effectiveness of the inoculation at different plant ages, roots of 13, 17, 21, 25 day-old plants were wounded with a longitudinal split and replanted into infested soil. Plants were maintained in greenhouse conditions and evaluated for foliar severity over time. In another experiment, 10, 15 and 20 day-old plants with wounded and non-wounded roots were introduced into a *F. virguliforme* cell-free toxin filtrate. Plants were maintained in growth chamber conditions, and foliar severity was evaluated over time. In soil assays, severity of foliar symptoms was similar in

split root and cut root methods, and was negatively correlated with plant age. In toxin assays, foliar severity did not differ among wounded and intact roots, but was greater ($P < 0.01$) in the 10-day old plants than in 15 or 20 day-old plants. Toxin assay with intact roots was identified as a simple and effective inoculation method for VIGS studies. We also revealed that soybeans become less susceptible to the *F. virguliforme* toxin as they mature, generating intriguing questions about the role of plant age on SDS.

Effect of planting density, SCN population, and soil pH on soybean root rot

K. Lim (1), C. GONGORA (1), P. Caragea (2), L. Leandro (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA; (2) Department of Statistics, Iowa State University, Ames, IA, USA
Phytopathology 100:S188

Root health is essential for crop growth and productivity, but the factors affecting root rot on soybeans are poorly understood. The objective of this study was to investigate the effect of planting density, SCN population, and soil pH on soybean root rot and yield. Field studies were established in 2006 and 2007 following a split-plot design with row spacing (15" and 30") as the main plot and plant population density (100K, 125K, 150K, 175K and 200K seeds/acre) as the split plot. Soil pH and SCN density were assessed in each of the 80 field plots. Roots collected at flowering were assessed for root rot severity, root dry weight and colonization by fungal pathogens. Yield was determined. Regression analysis was conducted accounting for spatial dependence between the variables. A clustered ($P < 0.01$) spatial pattern was found for pH, SCN, root rot and dry weight in 2006 and 2007, and for yield in 2007. Soil pH and SCN showed a similar spatial pattern in the field as root rot severity. Plant population and row spacing did not affect root rot severity. Root dry weight was affected ($P < 0.05$) by row spacing and plant population in 2006, and by plant population in 2007. Soil pH was positively correlated with root rot severity ($r = 0.92$, $P < 0.001$) and ($r = 0.28$, $P = 0.02$), in 2006 and 2007 respectively, and negatively correlated with root dry weight ($r = –0.6$, $P < 0.001$) and yield ($r = –0.3$, $P = 0.07$) in 2007. SCN population was positively correlated with root rot ($r = 0.5$, $P < 0.05$) both years. Fusarium was the predominant fungus isolated from roots, and was more frequently isolated from roots with >30% root rot than roots with less severe root rot. This study suggests that soil pH plays an important role in soybean root rot and productivity. The interaction between soil pH and root pathogens warrants further research.

Quantifying and comparing the aggressiveness of *Pantoea stewartii* isolates from Iowa

L. LIU (1), C. C. Block (1), F. W. Nutter (1)
(1) Iowa State University, Plant Pathology Department, Ames, IA, USA
Phytopathology 100:S188

Stewart's disease, caused by *Pantoea stewartii*, can cause severe economic damage to seed and sweet corn crops due to phytosanitary regulations that prevent the export of seed, as well as cause direct reductions in yield. The aggressiveness of thirteen *Pantoea stewartii* isolates was quantified and compared by measuring incubation period (day), rate of lesion expansion/day, and time to leaf death. Growth chamber experiments were conducted at the optimal temperature of 30°C. Sweet corn plants (variety "Jubilee") were inoculated at the V8 growth stage with 12 wild-type *Pantoea stewartii* isolates and a rifampicin-naldixic acid resistant isolate, Rif 9A. Both sides of the mid-rib of 4 leaves per plant were inoculated with 1 of the 13 isolates (1×108 CFU/ml). There were 5 corn plants for each isolate and 65 plants per replication. Experiments were performed twice for each isolate. Acropetal and basipetal lesion expansions were measured beginning when lesions were first visible. Measurements continued at 24-h intervals until no further lesion expansion was possible (leaves were dead). Our results to date show no statistical difference among lesion expansion rates of the 13 *Pantoea stewartii* isolates, which averaged 0.3984 cm/day acropetally and 0.4999 cm/day basipetally. Of the 4 leaves tested, average expansion rates were fastest (0.6018 cm/day acropetally and 1.0804 cm/day basipetally) on the eighth true leaf. Incubation period was shortest on the seventh true leaf (7.7831 days). There was no statistical difference between acropetal and basipetal expansion rates. This study, the first to quantify the aggressiveness of *Pantoea stewartii* isolates, serves as a baseline for detecting shifts in pathogen aggressiveness.

Effect of co-inoculation of *Fusarium virguliforme* and *Phialophora gregata* on soybean

C. MATTUPALLI (1), P. D. Esker (1)
(1) University of Wisconsin, Madison, WI, USA
Phytopathology 100:S188

Fusarium virguliforme (*Fv*, causal agent of sudden death syndrome, SDS) and *Phialophora gregata* genotypes A and B (*PgA* and *PgB*, causal agent of brown stem rot, BSR) are two yield-limiting, soil-borne pathogens for Midwest soybean producers. To evaluate the possible interactions between *Fv*, *PgA*, and *PgB* on disease development, a greenhouse study was conducted. Two soybean cultivars, Jack (resistant to *Fv* and *PgA*) and Williams82 (susceptible to *Fv* and *PgA*) were planted in metromix+peat growth medium that was amended with pathogen-infested vermiculite. There were eight inoculum treatments: noninfested controls, *Fv*, *PgA*, *PgB*, *Fv+PgA*, *Fv+PgB*, *PgA+PgB*, and *Fv+PgA+PgB*. Individual pathogens were added in equal parts to yield 10,000 spores cm^{-3} of plant growth medium. Foliar symptoms characteristic of either SDS or BSR were assessed during reproductive stages (R1-R7) as the percentage of plant area infected. Mean area under disease progress curve (AUDPC) ranged from 0%·days for noninfested controls to 518.84%·days for Jack inoculated with *Fv*. Results indicated that there was an effect of variety ($P < 0.0001$), inoculum ($P = 0.0092$) and their interaction ($P = 0.0756$). Multiple comparisons using a Tukey adjustment suggested that Jack inoculated with *Fv+PgA* had greater disease development compared with all Williams82 inoculum treatments. These preliminary results suggest that *Fv* and *PgA* interact with each other and that their effect varies between cultivars.

First report of *Fusarium* root rot in soybean caused by *Fusarium tricinctum* in Minnesota

P. W. Meyer (1), M. S. Clancey (1), I. E. Brose (1), J. E. KURLE (1)
(1) Dept. of Plant Pathology, University of Minnesota, St. Paul, MN, USA
Phytopathology 100:S188

Seed, seedling, and root rots of soybean caused by a complex of soilborne fungi are possibly the most important diseases of soybean in Minnesota, causing losses estimated at 380,000 tons in 2005. *F. solani* and *F. oxysporum* are the predominant *Fusarium* species isolated from soybean taproots in Minnesota. For soybeans grown in unamended field soil in a growth chamber at 10 and 16°C, the predominant *Fusarium* species isolated from taproots were *F. solani* and *F. tricinctum*. Three isolates of *F. tricinctum* were obtained from these plants. One of the isolates produced lesions on soybean seedlings after two weeks, using an inoculum layer method in inoculated sterile sand. *F. tricinctum* has been previously reported as pathogenic on soybean in Ontario, Canada. Its preference for lower temperatures might account for the low frequency of isolation from Minnesota grown soybean. Its role in soybean root rot in the field is not known. *F. tricinctum* could contribute to seedling rot early in the season when the soil temperature is below 20°C.

Differential regulation of host mRNA translation initiation in the *Arabidopsis*: TuMV interaction

J. MOELLER (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 100:S188

Viruses are known for their ingenuity in reprogramming the host processes of transcription and translation, including use of non-canonical methods of translation initiation. To assess virus-induced changes in host transcription and translational processes, we used the *Arabidopsis* ATH1 GeneChip oligonucleotide microarray to determine the mRNA species bound to 80S ribosomes versus the mRNA species present in total RNA populations in the *Arabidopsis*:Turnip mosaic virus (TuMV) interaction. The majority of genes that are either well or poorly loaded onto ribosomes are consistent in their loading behavior between non- and TuMV-infected tissues. However, considerable differential regulation of translation initiation was also found when non- and TuMV-infected tissues were compared. For example, there are numerous genes that are up-regulated upon infection according to their mRNA abundance in total RNA populations but show down-regulation according to the genes' translation initiation status and vice-versa. In support of this finding, 1071 probe sets showed over 4-fold difference when contrasting mRNA from total RNA to mRNA from 80S ribosomes in response to TuMV. This study provides near genome-scale analysis of the regulation of translation initiation in both non- and TuMV-infected states, and it suggests that analyses of mRNA abundance in total RNA may lead to incorrect conclusions about which genes are induced or down-regulated in response to viral infection. Because mRNA associated with 80S ribosomes is expected to be more predictive of the proteome, this approach may provide candidate genes with greater relevance to the *Arabidopsis*:TuMV interaction.

Engineering payload designs for remote sensing applications for plant pathology using latex weather balloons

M. E. NELSON (3), J. P. Basart (2), F. W. Nutter, Jr. (1)
(1) Department of Plant Pathology, (2) Electrical and Computer Engineering Department, (3) Space Systems and Controls Lab, Aerospace Engineering Department, Iowa State University, Ames, IA, USA
Phytopathology 100:S188

The High Altitude Balloon Experiments in Technology (HABET) program at the Space Systems and Controls Lab (SSCL) at Iowa State University (ISU) has been flying high altitude balloons in collaboration with the ISU Department of Plant Pathology for over 10 years. Project goals are to obtain real-time imagery of crops under stress from biotic and abiotic agents, as well

as to quantify the density of pathogen spore clouds above diseased crops. These flights vary from ground tethered flights to flights reaching altitudes of 30 km or higher. Since 2007, the two teams have been working together to design, build and fly hardware that is capable of acquiring both visible and near-infrared digital images. The engineering design of such hardware presents unique opportunities in building robust, yet accurate and reliable equipment for the detection and accurate identification of various plant diseases. The hardware we are using consists of 2 Digital SLR cameras (Canon 5D cameras with 24-105 mm zoom lenses). However, one of the Canon 5D cameras has been modified for near-infrared operation in the 830 nm near-infrared range. A Single Board Computer is used to remotely control the cameras through a USB connection and allows us to take photographs as well as adjust camera settings while the payload is in the air. The helium balloon platform has also been used to quantify the horizontal and vertical gradients of spore densities being released from disease plant canopies. We have designed and built payloads that are capable of flying 6 Model 20 Rotorod spore collectors which are also remotely controlled from a ground control station. This system allows spore collection at 6 different altitudes to obtain a vertical profile of spore densities. Flights in the near future are being planned for balloons to be released at pre-set altitudes to quantify spore densities horizontally with respect to distance from the source (diseased field).

Effect of sclerotial moisture content on carpogenic germination of *Sclerotinia sclerotiorum*

A. NEPAL (1), L. E. del Rio (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 100:S189

The effect of sclerotial hydration levels on *Sclerotinia sclerotiorum* carpogenic germination (CG) was studied under controlled environment. Sclerotia of *S. sclerotiorum* isolate WM031 was classified as large, medium or small by sieving. Sclerotial water uptake in plain water and in three soil textures set at four water content levels was characterized using four replications and ten sclerotia per replication. Sclerotia were placed on petri dish bottoms in moist chambers that kept them at 100%, 70–80%, 40–50%, and 20–30% of their maximum hydration level using cool mist humidifiers. Moist chambers were set at 18/14°C day/night for three months prior to CG quantification. The experiment was replicated three times with 15 sclerotia per replication. Water uptake rate by small sclerotia was significantly higher (alpha = 0.05) than medium and large sclerotia in all moisture treatments. Small sclerotia were fully hydrated in <5 hours, medium sclerotia in <15 hours, and large sclerotia in <25 hours, irrespectively of the texture and saturation levels of the soil in which they were incubated. A significant interaction (alpha = 0.05) between sclerotial hydration level and size was observed for both, CG and the average number of apothecia produced per sclerotium. At 100% hydration, large sclerotia had 1.7 and 2.9 times more CG and apothecia per sclerotium, respectively, than medium and small sclerotia. At 70 to 80% hydration, only 10% of medium and small sclerotia produced apothecia while large sclerotia did not produce any. Sclerotia at 50% hydration or drier did not produce apothecia irrespectively of size.

Is *Rps*8 alone? Evidence for different genes for resistance to *Phytophthora sojae* on the chromosome 13 of soybean PI 399073

M. A. ORTEGA (3), D. Tucker (1), S. A. Berry (3), S. St. Martin (2), S. Maroof (1), P. Cregan (6), D. Hyten (4), R. Shoemaker (5), A. E. Dorrance (3)
(1) Department of Crop and Soil Environ. Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA; (2) Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH, USA; (3) Department of Plant Pathology, The Ohio State University, Wooster, OH, USA; (4) SDA ARS, Soybean Genomics and Improvement Lab. BARC, Beltsville, MD, USA; (5) USDA ARS, Department of Agronomy, Iowa State University, Ames, IA, USA; (6) USDA ARS, Soybean Genomics and Improvement Lab. BARC, Beltsville, MD, USA
Phytopathology 100:S189

PI 399073, a plant introduction from South Korea, is the source of *Rps*8, one of the genes that confers resistance to *Phytophthora sojae*, the causal agent of Phytophthora root and stem rot in soybeans. Three Williams (*rps*8/*rps*8) × PI 399073 (*Rps*8/*Rps*8) $BC_4F_{2:3}$ populations were evaluated for the introgression of *Rps*8 by association of resistance to *P. sojae* race 25 (1a, 1b, 1c, 1k, 7) with 72 SSR and SNP markers from a region on chromosome 13 where *Rps*8 was previously mapped. A PI 399073 (*Rps*8/*Rps*8) × PI 408211B (*Rps*?/*Rps*?) $F_{3:4}$ population was used to map resistance to *P. sojae* isolate Butmu (1a, 1b, 1k, 2, 7), and was positioned below the introgression site found in the backcrosses. Williams (*rps*8/*rps*8) × PI 399073 (*Rps*8/*Rps*8) $BC_4F_{4:5}$ lines, each line having different location and size of introgression in this region of chromosome 13, were inoculated with the isolates of race 1, 4, 7, 17, 25, and Butmu. The phenotypes of each line were different from each other for the same isolate, as well as to different isolates, this could be attributed to different *Rps* genes present in PI 399073 that were introgressed differentially in the lines tested. The position and size of the introgression on chromosome 13 in a particular $BC_4F_{4:5}$ line could carry one or more *Rps* genes, the response to a different pathogen isolate could depend on which gene or genes were present in that line. These results suggest that PI 399073 could have two or more resistance to *Phytophthora sojae* genes on chromosome 13.

Aggressiveness of different *Fusarium graminearum* chemotypes on wheat cultivars with different level of resistance to Fusarium head blight

K. D. PURI (1), S. Zhong (1)
(1) North Dakota State University, Fargo, ND, USA
Phytopathology 100:S189

Fusarium head blight (FHB), caused by *F. graminearum* Schw., is a destructive disease of wheat and barley throughout the world. The disease is responsible for both direct yield reduction and mycotoxin contamination of grains. The major mycotoxins produced by the pathogen include deoxynivalenol (DON) and its derivatives [3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON)] as well as nivalenol (NIV), which pose health hazards to human and animals. The relative aggressiveness of 132 isolates collected during 1980 to 2000 in North Dakota, 43 isolates collected in 2008 from different counties of North Dakota and 59 isolates from China were evaluated after their chemotype. PCR assay indicated that 124 (93.9%) isolates from the old collection (1980 to 2000) and 24 (55%) from the new collection (2008) were of 15-ADON chemotype, and 46 (77.9%) from China were of NIV chemotype. Fourteen isolates from each of 15-ADON and 3-ADON chemotypes, and two from the NIV chemotype were tested for aggressiveness on three wheat cultivars/line (Grandin, Steele-ND and ND 2710), which are susceptible, moderately susceptible and moderately resistant to FHB, respectively. Mean disease severity induced by the isolates varied from 13.5 to 55.6% and difference in aggressiveness among isolates were highly significant (P = 0.0001). Majority of 3-ADON producing isolates had higher disease severity compared to 15-ADON or NIV isolates, but no isolate/variety interaction was detected. The results indicate that the 3-ADON chemotype isolates of *F. graminearum* have increased in the population of North Dakota and in general were more aggressive than 15-ADON and NIV isolates.

Comparison of molecular and mycelium assay for determining benzimidazole resistance in field populations of *Venturia inaequalis* in Indiana

K. L. QUELLO (1), K. Chapman (1), J. Beckerman (1)
(1) Purdue University, West Lafayette, IN, USA
Phytopathology 100:S189

Apple scab, caused by the fungus *Venturia inaequalis*, is the most destructive disease on apples in the Midwest, and is controlled primarily by fungicides. As a result, fungicide resistance has become a problem in orchards. Fungicide resistance testing requires pure cultures of the fungus. Unfortunately, isolating pure cultures of V. inaequalis after the end of spring is difficult due to the microflora on the apple leaf. The use of a molecular assay in situ could avoid this requirement. We developed a screen that utilizes PCR in situ to detect Topsin-M (thiophanate-methyl) resistance. *V. inaequalis* isolates collected from Indiana were screened with mycelium assays for thiophanate-methyl resistance. Isolates were found to range from sensitive (no growth at 0.5 µg active ingredient (a.i.) thiophanate-methyl /mL) to low resistance (growth at 0.5 µg a.i./mL but not 5 µg a.i./mL) to medium resistance (growth at 5 µg a.i./mL but not at 50 µg a.i./mL) to very high resistance (rapid growth at 50 µg a.i./mL). To test the accuracy of a molecular assay, concordance between known mutations in the beta-tubulin gene and phenotype was determined. DNA was extracted from pure cultures and the beta-tubulin gene was amplified and digested. Restriction enzyme BstUI was used to verify a restriction fragment length polymorphism (RFLP) at codon 198 that corresponded to very high fungicide resistance. 68% of resistant isolates were positive for the polymorphism. The remaining resistant isolates that did not contain the RFLP were sequenced. 100% of these isolates possessed a point mutation at codon 240 in the beta-tubulin gene. This mutation can be differentiated by PCR-RFLP using Cac8I. All resistant isolates could be identified with the two restriction enzyme digests. These two PCR-based RFLP detection methods could be used to rapidly detect thiophanate-methyl resistance isolates of *V. inaequalis* at any time.

Extended-duration row covers to suppress bacterial wilt on muskmelon: Optimizing a new management strategy

E. SAALAU ROJAS (1), M. L. Gleason (1), J. C. Batzer (1)
(1) Iowa State University, Ames, IA, USA
Phytopathology 100:S189

Bacterial wilt (pathogen: *Erwinia tracheiphila*) causes major losses on muskmelon in the Midwest U.S. Extending the period during which plants are covered by spunbond row covers may shield crops from cucumber beetles, which vector the pathogen. Experiments at two Iowa State University research

farms (Muscatine and Gilbert, IA) in 2008 validated the ability of extended-duration row covers to suppress incidence of bacterial wilt on muskmelon. Treatments in a latin square design were: 1) no row cover; 2) row cover removed at the beginning of anthesis (start of bloom); 3) row covers removed 10 days after anthesis, with row cover ends opened at anthesis to allow pollination; and 4) row covers removed 10 days after anthesis, with bumble bee boxes inserted under row covers at anthesis to provide pollination. In both trials, wilt incidence in the non-covered control was much higher than in the row-covered treatments. Yield in the extended-duration row cover treatments was similar when row ends were opened or when a bumble bee box was inserted under the cover. At Muscatine, the extended-duration row covers significantly reduced incidence of bacterial wilt at harvest compared to row cover removal at anthesis. At Gilbert, where melons were transplanted 3 weeks later than at Muscatine, all row cover treatments resulted in similar levels of wilt suppression. The results suggest that row covers can effectively suppress cucurbit bacterial wilt, and that timing of transplanting may determine whether extending the row-covered period provides an additional margin of wilt protection.

Pathotype diversity of *Phytophthora sojae* plant isolates from Iowa

S. M. STEWART (1), A. E. Robertson (1)
(1) Department of Plant Pathology, Iowa State University, Ames, IA
Phytopathology 100:S190

Phytophthora root rot (PRR) caused by *Phytophthora sojae* can infect soybeans at all growth stages, causing pre- and post-emergence damping-off and root and stem rot. The most effective way to manage PRR is through the use of *P. sojae*-resistant cultivars however, the pathogen continues to diversify and overcome resistant genes (Rps) present in commercial cultivars. This host-pathogen system follows Flor's gene-for-gene hypothesis, and there are 13 known Rps genes. Pathotype diversity has been monitored in Iowa since 1966. Prior to 1975, race 1, which is capable of defeating the resistance gene Rps7, was the only pathotype reported in Iowa however, two decades later 100% of isolates of *P. sojae* recovered from soybean plants were able to infect plants with Rps7. Since only 4.6% of isolates of *P. sojae* in 1976 were able to infect plants with Rps-1k, soybean cultivars with Rps-1k were marketed commercially for PRR management, but by 2004, 73.3% of isolates of *P. sojae* recovered from soybean plants could infect plants with Rps-1k. In 2008, the pathotype diversity of 41 isolates of *P. sojae* recovered from 15 soybean plants sampled from six commercial fields in Iowa was assessed using 14 differentials. The isolates belonged to six unique pathotypes. In four fields, only one unique pathotype was recovered from the plants sampled, while in the other two fields, two unique pathotypes were recovered. In the study, 100% of the isolates were able to infect plants with Rps-7 and 85.4% could infect plants with Rps-1k. As expected, the endemic *P. sojae* population in Iowa continues to diversify and selection pressure posed by commercial *P. sojae*-resistant cultivars results in a greater number of isolates compatible on these cultivars.

Effectiveness of Brassica short-cycle cover crops in managing *Phytophthora capsici* and *Fusarium* spp. in cucurbit fields

S. THRU PPOYIL (1), M. Babadoost (1)
(1) UIUC, Urbana-Champaign, IL, USA
Phytopathology 100:S190

A study was conducted in 2008 to determine effectiveness of *Brassica* short-cycle cover crops in managing *Phytophthora capsici* and *Fusarium* spp. in cucurbit fields. Mustard cultivars, Florida Broadleaf (FBL) and Tilney were seeded on 29 April in a field with a history of Phytophthora blight and Fusarium fruit rot of pumpkins and watermelon. The mustard crops were grown for 45 days and then incorporated into top 10-cm layer of the soil after cutting the mustard plants with a disk cultivator. Jack-o-Lantern pumpkin 'Magic Lantern', processing pumpkin 'Dickinson', and cucumber 'Eureka' were grown in the mustard amended area. Incidence and severity of seedling death, leaf spot, vine infection and fruit rot caused by *P. capsici and Fusarium* spp. were assessed on a biweekly schedule starting from seedling emergence on 14 July until harvest on 26 September. No seedling infection or leaf spot were observed in the plots. Percentage of vines infected with *P. capsici* in the plots on 19 September were 16.0, 22.5, 23.0, and 27.5% in the plots amended with FBL, Tilney, FBL+Tilney, and control plots, respectively. Similarly, percentage of fruits infected with *P. capsici* on 26 September were 32.2, 33.5, 33.6, and 42.4% in plots amended with FBL, Tiney, FBL+Tilney, and control plots, respectively. No Fusarium infection was detected in the plots.

Virulence and genetic diversity of *Phakopsora pachyrhizi* in Nigeria

M. TWIZEYIMANA (2), P. Ojiambo (4), J. Haudenshield (1), G. Caetano-Anollés (1), K. Pedley (5), R. Bandyopadhyay (6), G. Hartman (3)
(1) Dept. of Crop Sciences, University of Illinois, IL; (2) Dept. Crop Sciences, University of Illinois, IL and International Institute of Tropical Agriculture (IITA), Nigeria; (3) Dept. of Crop Sciences, University of Illinois and USDA-ARS; (4) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC; (5) Foreign Disease-Weed Science Research Unit, USDA-ARS, Fort Detrick, MD; (6) International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
Phytopathology 100:S190

Soybean rust, caused by *Phakopsora pachyrhizi*, is a major disease in many soybean-producing areas in Nigeria. To determine the virulence and the genetic structure of Nigerian field populations of the soybean rust pathogen, a total of 116 purified isolates established from infected leaves randomly collected from soybean fields in four agroecological zones in 2005 was used. The virulence variability of the isolates was determined using a set of four soybean accessions with Rpp1, Rpp2, Rpp3, and Rpp4 resistance genes, two highly resistant and two highly susceptible genotypes. Principal component and cluster analyses on the number of uredinia per cm2 of leaf tissue separated the rust isolates into seven pathotype clusters. Isolates in cluster III were the most virulent, while those in cluster IV were the least virulent. In a follow-up study, 18 simple sequence repeat markers were used to study the genetic diversity using the same116 isolates and an additional 146 isolates collected from infected plants in two fields (73 isolates in each field) located 292 km apart. There was a high genetic variation in Nigerian *P. pachyrhizi* populations. Eighty-four distinct genotypes were identified among isolates from the three agroecological zones, while 48 distinct genotypes were identified from 146 isolates analyzed from both fields. Nei's average genetic diversity across geographical regions was 0.22 while for both fields was 0.09. Hierarchical analysis of molecular variance revealed significant ($P < 0.05$) and low genetic differentiation among all populations of *P. pachyrhizi*. However, the majority (> 90%) of the genetic diversity was distributed within a soybean field, while almost 6% was distributed among fields within geographic regions. The phylogenetic analysis showed three groups in Nigerian rust populations with one major group comprising more than 90% of the isolates. However there was a poor correlation between virulence and genetic variation. This work will be useful in breeding and management of soybean rust by facilitating the deployment of rust-resistant cultivars.

Performance of SCN-resistant soybean varieties in fields infested with different soybean cyst nematode HG types

G. L. TYLKA (1), G. D. Gebhart (1), C. C. Marett (1)
(1) Iowa State University, Department of Plant Pathology, Ames, IA, USA
Phytopathology 100:S190

There are hundreds of soybean varieties resistant to the soybean cyst nematode (SCN). These varieties vary in yield and the ability to control SCN populations. The HG type test is a greenhouse test that assesses SCN reproduction on the different sources of resistance used in breeding SCN-resistant soybean varieties. Each year, we evaluate the agronomic performance and SCN control of SCN-resistant soybean varieties in field experiments, and results reveal how the HG type of an SCN population relates to performance of SCN-resistant soybean varieties in the field. There are nine experimental locations statewide annually, three each in northern, central, and southern Iowa. Plots are four rows wide, spaced 76 cm (30 inches) apart and 5.2 meters (17 feet) long. Each variety is replicated four times per location. Soil samples are collected from each plot at planting to verify the presence of SCN and to determine the initial SCN population density. Also, an HG type test is conducted on the SCN population obtained from the spring soil samples at each location. At harvest, another soil sample is collected from each plot to determine SCN population densities. The center two rows of each plot are harvested, and yield and SCN population densities are averaged for each variety at each location. The highest-yielding SCN-resistant varieties often are those with a source of resistance on which there was low (<5 percent) SCN reproduction in the HG type test. But in some experiments, the highest yielding SCN-resistant soybean varieties are those with a source of resistance on which there was relatively high (>20 percent) SCN reproduction in the HG type test. Also, in some experiments, SCN population densities declined or did not increase during the growing season on varieties with sources of SCN resistance on which there was >20% reproduction in the HG type test.

Discovery of genes underlying soybean QTLs conferring partial resistance to *Phytophthora sojae*

H. WANG (4), L. Waller (5), S. Tripathy (5), S. K. St. Martin (3), L. Zhou (5), K. Krampis (5), D. M. Tucker (1), I. Hoeschele (2), S. Maroof (1), B. Tyler (5), A. E. Dorrance (4)
(1) Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA; (2) Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA; (3) The Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH, USA; (4) The Department of Plant Pathology, The Ohio State University, Wooster, OH, USA; (5) Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
Phytopathology 100:S190

Phytophthora sojae causes soybean root and stem rot, resulting in an annual loss of 1–2 billion dollars in soybean production worldwide. Partial resistance confers a broad-spectrum durable resistance to *P. sojae* and is currently thought to be a more stable alternative than single gene mediated resistance. Few QTLs have been mapped for soybean partial resistance to *P. sojae* and little is known about the molecular mechanisms behind it. In this study, five potential QTLs on Chromosomes 12, 13, 14, 17 and 19, each explaining 4–7% of phenotypic variation, were identified from 186 RIL of a $F_{4:7}$ population from a cross of the partially resistant cultivar 'Conrad' and the susceptible cultivar 'Sloan' by composite interval mapping. Global expression profiling identified a large number of genes showing expression contrast between 'Conrad' and 'Sloan' either after inoculation or constitutively. Of these, 55 genes map to the QTL regions and include defense-related proteins such as auxin response factors, heat shock proteins, transcription factors, membrane transporters, NBS-LRR proteins, pyruvate decarboxylase, cytochrome P450, cysteine protease and H(+)/calcium ATPase. Eighteen of the 55 (32.7%) proteins are either unknown or have uncharacterized functions. Fifteen genes under QTLs were selected and their expression was confirmed by qRT-PCR. The results indicate the possibility of a complex QTL-mediated resistance network and provide the clues for further functional studies of soybean partial resistance to *P. sojae*. These genes could also be used as markers for breeding and thus improving soybean production.

Field and greenhouse evaluation of fungicide seed treatment control of sudden death syndrome of soybean

J. D. WEEMS (2), G. Zhang (2), K. A. Ames (2), J. P. Bond (1), C. A. Bradley (2)
(1) Southern Illinois University, Carbondale, IL, USA; (2) University of Illinois, Urbana, IL, USA
Phytopathology 100:S191

Sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is a yield reducing disease common in many soybean producing states. Results of recent research indicated that infection can occur during early radicle emergence, suggesting fungicide seed treatments may provide protection from the pathogen during the early stages of soybean development. In 2008, a field study across two locations and a greenhouse study were conducted to test eleven fungicide seed treatments and an untreated control across four cultivars for effects on *F. virguliforme* infection and development. The southern Illinois location (Valmeyer) was naturally infested with *F. virguliforme*, the central Illinois location (Urbana) was naturally infested with *F. virguliforme* and soil was augmented with sterilized grain sorghum colonized by *F. virguliforme*, and the greenhouse study was artificially infested with *F. virguliforme* inoculum. Roots collected from plots were scanned and analyzed using WinRHIZO. Foliar symptoms of SDS were rated during plant growth and harvest data were collected to monitor disease development. At Valmeyer, SDS was most prevalent and seed treatments had a significant ($P = 0.0002$) effect on early season plant stand, with the untreated control having the lowest stand. Furthermore, roots from untreated plots collected from Valmeyer had significant root tip reductions ($P = 0.0052$) and increased average root diameter ($P = 0.0451$), suggesting lateral root and root hair reduction. Seed treatments had no other significant effect on the pathogen or disease development.

The 2007 and 2008 Fusarium head blight epidemics in Nebraska

S. WEGULO (1), P. Baenziger (1), L. Nelson (1), J. Hernandez Nopsa (1), J. Millhouse (1), N. Mengistu (1), J. Breathnach (1)
(1) University of Nebraska-Lincoln, Lincoln, NE, USA
Phytopathology 100:S191

Because of a variable climate, including drought during some years, *Fusarium* head blight (FHB) occurs sporadically in Nebraska. In 2007 and 2008, FHB epidemics occurred in the state for the first time in more than a decade. Infection of wheat heads by *Fusarium graminearum* was favored by excessive rainfall before and during flowering. The most affected areas were the south central and eastern parts of the state. However, in 2008, FHB was observed as far west as Imperial in the southwestern part of the state where irrigated fields were more severely affected. Northwest, the Nebraska Panhandle was spared in both years due to dry conditions. A shift towards reduced tillage or no-till to conserve water and soil and inclusion of corn and wheat in crop rotation schemes has led to buildup of FHB inoculum in Nebraska. Yields were reduced not only by FHB but by other foliar diseases favored by wet weather. The major foliar diseases were *Septoria tritici* blotch, powdery mildew, and tan spot. In addition to reducing yield and grain quality, FHB caused accumulation of the mycotoxin deoxynivalenol (DON) in grain. Yield losses of up to 20% were estimated in the most severely affected areas in the south central and eastern parts of the state. In 2007, the overall loss statewide in grain yield was estimated at 2.0% or 1.68 million bushels valued at $9.4 million based on a June 11, 2007 wheat price of $5.57/bushel. In 2008, the overall loss statewide was estimated at 2.3% or 1.64 million bushels valued at $13.3 million based on an August 28, 2008 wheat price of $8.11/bushel. Additional losses were incurred in reduced prices for the infected grain with high levels of DON. In the most severely affected areas in both years, DON concentrations of more than 18 ppm were recorded in the most susceptible cultivars.

Detection of *Melon necrotic spot virus* in *Olpidium* sp. infested cucumbers

C. D. WOLTJEN (1), D. J. Lewandowski (1)
(1) The Ohio State University, Columbus, OH, USA
Phytopathology 100:S191

Melon necrotic spot virus (MNSV) is an important pathogen of cucumbers and melons that can lead to a reduction in fruit quality and economic losses. MNSV is vectored by zoospores of *Olpidium* sp. and can also be mechanically transmitted. In 2008, we received a call from a greenhouse cucumber grower in Ohio with a high percentage of symptomatic cucumber *(Cucumus sativus)* plants exhibiting large necrotic foliar lesions. Leaf samples from symptomatic cucumbers tested positive for MNSV by DAS-ELISA. Roots of symptomatic plants were found to be infested with *Olpidium* sp. The objectives of our research were to compare the sequence of this Ohio MNSV isolate to other known MNSV isolates and to determine the susceptibility of several cucurbit species. Sap from MNSV-infected cucumber leaves was rub-inoculated onto the cotyledons of three *C. melo* and seven *C. sativus* cultivars. Necrotic local lesions on cotyledons of all ten cultivars were confirmed to be MNSV-positive by DAS-ELISA. MNSV was also transmitted from the original *Olpidium* sp. infested substrate to one *C. sativus* variety. cDNA was synthesized from total RNA extracted from MNSV-infected leaves using an MNSV primer complementary to the 3′-UTR. The coat protein (CP) ORF was amplified with PCR using the same 3′ primer and a primer located upstream of the CP ORF. The gel-purified PCR product was sequenced and compared to other known isolates of MNSV. The CP of the Ohio MNSV isolate is 84–94% and 74–97% identical to the other isolates of MNSV at the nucleotide and amino acid levels, respectively.

Bacterial species associated with internally-discolored horseradish roots

J. YU (1), M. Babadoost (1)
(1) University of Illinois, Urbana, IL, USA
Phytopathology 100:S191

Internal discoloration of horseradish roots is a complex disease, caused by at least three fungal pathogens, *Verticillium dahliae, V. longisporum,* and *Fusarium solani*. In addition to the fungal species associated with internally discolored horseradish roots, bacteria have been routinely isolated from the affected roots. This study was conducted to identify bacterial species associated with internally discolored horseradish roots. Horseradish root samples were collected from major horseradish growing areas in North America, including Illinois, Wisconsin, California, and Ontario (Canada), and were assayed for presence of bacteria. The outer layer of the diseased roots were peeled and surface sterilized in a 6% sodium hypochlorite solution for 1 minute, followed by a 95% ethanol concentration for 3 minutes, and then rinsed in sterile-distilled water three times. Five segments from each root were placed onto nutrient agar (NA) plates. The plates were incubated at 22–28°C with 12 h light/12 h darkness. Bacterial growth were observed after 5, 10, 15 days of incubation. Single-cell colonies of each isolated bacterium were grown on NA. Characteristics of each purified colony were recorded. Isolated bacteria were identified using the Biolog program and polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay followed by analyzing 16s rDNA sequences. *Pseudomonas fluorescence, Bacillus cereus*, and *Erwinia* spp. were the main bacterial species isolated from horseradish root samples.

Genetic variation of *Phytophthora sojae* populations from Ohio and South Dakota

L. X. ZELAYA-MOLINA (1), M. A. Draper (2), A. E. Dorrance (1)
(1) Dept. of Plant Pathology, OARDC, Wooster, OH; (2) formerly Plant Science Department, South Dakota State University, Brookings, SD
Phytopathology 100:S191

Phytophthora sojae is an important plant pathogen of soybean and negatively impacts yield each year in the north central region. Understanding how the pathogen population is evolving will assist in developing effective management strategies. Our objective was to assess the population variation of *P. sojae* isolates collected from 2 states, OH and SD and 2 intensively sampled fields within OH, Sandusky and Wood, with 21 polymorphic SSR markers. The average number of alleles was 3.6 per loci for the OH population, and 2 of the 32 isolates were putative heterozygotes for 2 SSRs. Seventeen alleles were exclusive to the OH population. SD population had fewer alleles, 2.9; while one isolate was heterozygous for one SSR, and 4 alleles were exclusive. The Sandusky and Wood populations had an average of 3.1 and 3.3 alleles, respectively; Wood population had two heterozygous isolates. Nei's genetic distance and Fst analysis indicates a moderate genetic differentiation between the populations. The results agree with previous

reports of low level of outcrossing in these populations that could account for the generation of different pathotypes in the field.

Mapping multiple novel resistance genes against *Phytophthora sojae* in soybean PI 408211B

Z. ZHANG (2), S. A. Berry (2), R. Mian (3), S. K. St. Martin (1), A. E. Dorrance (2)
(1) Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH, USA; (2) Department of Plant Pathology, The Ohio State University/OARDC, Wooster, OH, USA; (3) USDA-ARS and Department of Horticulture and Crop Science, The Ohio State University/ORADC, Wooster, OH, USA
Phytopathology 100:S192

Fourteen *Rps* genes conferring resistance to *Phytophthora sojae*, which causes Phytophthora root and stem rot, have been identified in different soybean cultivars and plant introductions (PI). PI 408211B was proposed to have one novel dominant resistance gene against *P. sojae* race OH17 (*vir*1b, 1d, 2, 3a, 3b, 3c, 4, 5, 6 and 7) and three previously documented dominant resistance genes against race OH25 (*vir*1a, 1b, 1c, 1k and 7). Simple sequence repeat (SSR) and single-nucleotide polymorphisms (SNPs) markers tightly linked with the resistance to OH17 were identified. Using the soybean sequence assembly, new SSR and SNPs markers were developed to fine map this gene on a mapping population of 79 F4:5 recombinant inbred lines from a cross, 'Williams' X PI408211B. The gene was mapped between Scf260-027 and Satt530 with a map distance of 1.3cM and 4.9cM, respectively, on chromosome 3. The result was validated in an independent population of 48 F2:4 lines from a cross 'Sloan' X PI408211B. A population of 360 BC4:7 lines from backcrosses of 'Stressland' X PI408211B which does not have a locus for resistance to OH17 but maintained resistance to OH25 was used to map the resistance gene against OH25. Bulk segregant analysis (BSA) was used to screen 177 polymorphic SSR markers on twenty chromosomes with 10 to 20 cM intervals. None of the SSR markers previously linked with known Rps genes was associated with the resistance to OH25, which suggests that this resistance occurs at novel loci. BSA indicates that the resistance to OH25 is associated with chromosome 9 and upper part of chromosome 16. Single marker association analysis on 'Williams' X PI408211B F4:5 identified a region at lower half of chromosome 3 which was also associated with the resistance to OH25.

2009 Northeastern Division Meeting Abstracts

Abstracts presented at the APS Northeastern Division meeting in Quebec City, Canada, October 28–30, 2009. The abstracts are arranged alphabetically, by first author's name.

Rust diseases of cultivated turfgrasses: Understanding an old foe
L. A. BEIRN (1), J. Crouch (2), B. B. Clarke (1)
(1) Rutgers University, New Brunswick, NJ, USA; (2) USDA-ARS, Cereal Disease Laboratory, St. Paul, MN, USA
Phytopathology 100:S193

Rust is a common disease of cool-season turfgrasses that can decrease the aesthetic and economic value of many cultivated species, particularly Kentucky bluegrass (*Poa pratensis* L.). Chemical control of rust is costly and sometimes ineffective; therefore the use of resistant cultivars is important for the effective management of this disease. Over the past ten years, increased susceptibility to rust has been observed for several Kentucky bluegrass cultivars in the U.S., most notably the once highly resistant 'Midnight' types. It has been theorized that new races or even new species of the pathogen may be responsible for this shift in cultivar susceptibility, but the data needed to test this hypothesis is lacking. In the current study, we are using molecular markers to evaluate turfgrass rust populations. To date, 63 rust infested leaf samples have been obtained from graminicolous hosts in North America, the United Kingdom, Australia, and Chile. A reliable DNA extraction protocol was developed and both the complete internal transcribed spacer (ITS) region and 5.8S ribosomal DNA of the samples were amplified and sequenced. Assembled sequences ranged from 682 to 701 base-pairs in length, including the partial sequences of the flanking 18S and 28S rDNA. Bayesian phylogenetic analysis identified *Puccinia coronata*, *P. graminis*, and *P. striiformis* from infested samples, with *P. coronata* and *P. graminis* being most prevalent. Sequence data generated from this study has been used to design species-specific molecular markers to develop a real-time PCR protocol that can be utilized by turfgrass breeders, pathologists and diagnosticians for a quick identification of turfgrass rust species.

Compost amendment, a potential alternative to soil fumigation for the control of strawberry verticillium wilt
V. BERNIER-ENGLISH (1), T. J. Avis (2), B. Mimee (1), H. Antoun (1), R. J. Tweddell (1)
(1) Centre de recherche en horticulture, Pavillon de l'Envirotron, Université Laval, Québec, QC, Canada, G1V 0A6; (2) Department of Chemistry, Carleton University, Ottawa, ON, Canada, K1S 5B6
Phytopathology 100:S193

The application of certain composts is known to provide natural biological control against several diseases and appears as an interesting environmentally-respectful approach for the control of plant diseases. Verticillium wilt, caused by *Verticillium dahliae*, is an important disease affecting strawberry (*Fragaria* × *ananassa*). Currently, pre-plant soil fumigation with metham sodium (Vapam®) is commonly used to control the disease. However, Vapam fumigation implies serious risks for health and the environment and often leads to the eradication of beneficial organisms and to a negative shift in the biological equilibrium. The objective of the study was to evaluate the effect of compost application and Vapam fumigation on verticillium wilt incidence and on vegetative development and fruit yield of strawberry plants. Greenhouse and field assays have been conducted with three composts produced from either bovine manure, marine residues or forest bark residues. Composts were applied at different rates to *V. dahliae* naturally-infected field plots fumigated or non-fumigated with Vapam and planted with strawberries (cv. Seascape). In greenhouse assays, composts were applied to sandy substrate inoculated or non-inoculated with *V. dahliae* and planted with strawberries (cvs. Seascape and Chambly). The results indicate that incorporation of marine residues compost significantly increased vegetative development of strawberry plants in greenhouse and significantly reduced verticillium wilt incidence in the field. The results also indicate that soil fumigation significantly decreased fruit yield and total soil microbial biomass. Although fumigation significantly decreased soil populations of *V. dahliae*, it did not reduce verticillium wilt incidence.

Glyphosate effect on DON content and *Fusarium graminearum* inoculum production in wheat and barley
M. BÉRUBÉ (4), A. Vanasse (4), S. Rioux (1), N. Bourget (1), Y. Dion (2), G. Tremblay (2), G. Bourgeois (3)
(1) Centre de recherche sur les grains, Quebec City, Quebec, Canada; (2) Centre de recherche sur les grains, Saint-Mathieu-de-Beloeil, Quebec, Canada; (3) Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada; (4) Laval University, Quebec City, Quebec, Canada
Phytopathology 100:S193

Fusarium head blight (FHB) is an important disease of wheat and barley, particularly in the wet conditions of Eastern Canada. The principal pathogen associated with FHB, *Fusarium graminearum*, produces deoxynivalenol (DON), a mycotoxin that makes the grain unfit for food or feed. A recent survey conducted in eastern Saskatchewan revealed that glyphosate application in the previous 18 months within minimum-till system was significantly associated with higher FHB levels in wheat and barley. The objective of this study was to determine the glyphosate effect, used on soybean as the previous crop, on DON content and *F. graminearum* inoculum production in wheat and barley under three different tillage practices: conventional-till, minimum-till and no-till. Six field experiments (two species × three tillage practices) were conducted in Saint-Augustin-de-Desmaures and Saint-Mathieu-de-Beloeil in 2007 and 2008. Glyphosate and check herbicide treatments chosen according to weed species were applied as main plot treatments on RoundUp Ready™ soybean. The following year, three wheat and three barley cultivars of different FHB resistance levels were seeded as subplot treatments. In each main plot, two Petri plates containing a *Fusarium*-selective medium were placed facing the ground in order to capture spores coming from the previous crop. In 2007, there were no significant herbicide × cultivar interaction nor herbicide effects on DON content and inoculum production in any of the 12 experiments. In 2008, DON content was significantly ($P = 0.046$) enhanced by glyphosate use (1.5 vs 1.0 ppm) in only one trial of Saint-Augustin (barley, minimum-till), but there was no significant effect of glyphosate on *F. graminearum* inoculum production for any of the trials. Therefore, it seems that glyphosate used on soybean the previous year has no or low impact on DON content and *F. graminearum* inoculum production under Quebec cropping conditions, whatever the tillage practice used.

Fifty years of breeding for disease resistance in turfgrasses: Where we've been and where we're going
S. A. BONOS (1)
(1) Rutgers University
Phytopathology 100:S193

In the past fifty years, dramatic improvements have been made in breeding for disease resistance in cool-season turfgrasses. Significant breeding progress has been made for leaf spot (caused by *Drechslera poae*) and stem rust (caused by *Puccina graminis*) resistance in Kentucky bluegrass (*Poa pratensis*), gray leaf spot (caused by *Pyricularia grisea*) resistance in perennial ryegrass (*Lolium perenne*), brown patch (caused by *Rhizoctonia solani*) resistance in tall fescue (*Festuca arundinacea*) and colonial bentgrass (*Agrostis capillaris*), and dollar spot (caused by *Sclerotinia homoeocarpa*) resistance in creeping bentgrass (*Agrostis stolonifera*). There are some diseases for which significant improvements have not been made including red thread (caused by *Laetisaria fuciformis*) resistance in perennial ryegrass and pythium blight (caused by *P. aphanidermatum* and other *Pythium* spp.) in most cool-season turfgrasses. Historically, the dramatic improvements in disease resistance of the cool-season grasses have been attributed to traditional/conventional breeding techniques; however, it is likely that functional genomics and molecular techniques that identify specific genes and mechanisms involved in disease resistance will be significant in the development of cultivated turfgrasses in the future.

Evaluation of a dynamic model for primary infections caused by *Plasmopara viticola* on grapevine in Quebec

T. CAFFI (2), V. Rossi (2), O. Carisse (1)
(1) Agriculture and Agri-Food Canada, Horticultural Research Centre, 430 Gouin boul., Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6; (2) Istituto di Entomologia e Patologia vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy
Phytopathology 100:S194

A dynamic model for the prediction of *Plasmopara viticola* primary infections was evaluated by comparing model predictions with disease onset in 43 cases (locations per years) in Eastern Canada in 2008 and 2009. The model simulates the development of all oospore cohorts during the primary inoculum season, including: oospore germination; production and survival of sporangia; release, survival and dispersal of zoospores; infection and incubation. Bayesian analysis was used to evaluate the sensitivity, specificity and accuracy of the model predictions. First seasonal onset of downy mildew symptoms ranged between 8 June and 29 June depending year and vineyard. For each vineyard, one to 20 simulation runs were performed depending on the number of oospore cohorts formed, for a total of 545 simulations. All observed infections were correctly predicted by the model. A total of 313 simulations resulted in no infection and in 284 cases no disease developed. Only one observed infection was not predicted by the model. Finally, 29 out of 313 simulations predicted an infection that did not result in observed disease. From this validation analysis, it was concluded that this model could be used in Eastern Canada to predict the occurrence of the first infection and trigger the initiation of a fungicide spray program against grape downy mildew.

Age-related susceptibility of strawberry leaves and berries to infection by *Podosphaera aphanis*

O. CARISSE (1), J. Bouchard (2)
(1) Agriculture and Agri-Food Canada, 430 Gouin, St-Jean-sur-Richelieu, Qc, Canada, J3B 3E6; (2) Plant Science department, Laval University, Quebec City, Quebec, Canada, G1K 7P4
Phytopathology 100:S194

Powdery mildew, caused by *Podosphaera aphanis*, is a major disease of strawberry for which only few management tools are available. The importance of the disease varies with production systems (June bearing vs day neutral) which could be explained in part by the concurrent presence of susceptible leaves or berries and abundant airborne inoculum. Age-related susceptibility was studied by inoculating strawberry leaves and berries at different age group. The experiment was conducted for the June bearing cultivar 'Jewel' and the day neutral cultivar 'seascape'. On eight occasions in 2007, five plants for each cultivar were inoculated with dry conidia using a settling tower. They was a significant effect of leaf and berry age group on the susceptibility which decreased exponentially as leaves or berries aged to reach zero when the leaves were completely expanded or the berries at the pink stage at the time of inoculation. The proportion of maximum mildew severity as a function of leaf or berry growth stage was predicted using non-parametric regression (R^2 = 0.96 to 0.97). The prediction values were further validated with data collected in field naturally infected by *P. aphanis*. There was a linear relation between predicted and observed proportion of maximum mildew severity (R^2 = 0.95 to 0.98). The results of this study showed that timing fungicide sprays based on periods of high leaf and berry susceptibility should greatly improve management of strawberry powdery mildew.

First report of clubroot caused by *Plasmodiophora brassicae* on spring canola in Maine

S. B. JOHNSON (1), D. H. Lambert (1), P. J. Sexton (1)
(1) University of Maine, Orono, ME, USA
Phytopathology 100:S194

Spring canola (*Brassica napus* L.) was introduced into Maine as a potential oilseed crop in 1999. Since then, acreage has increased from 120 to about 3500 acres and is rapidly becoming an important rotation crop with potato. In August of 2008, canola plants in two fields in Aroostook County were observed with classical symptoms of clubroot. Severely affected plants were stunted and ripening prematurely. Many of the plants had the roots fully involved with the disease. Infected plants were collected from the fields. The roots were macerated and the resultant slurry allowed to settle. Abundant spores of *Plasmodiophora brassicae* were observed in the slurry. Spring canola was sown and inoculated with an excess of 50,000 spores per plant. A like number of spring canola plants were not inoculated. All canola plants were examined twelve weeks after inoculation. Typical root clubbing symptoms were evident in all of the inoculated plants. The roots were macerated and *Plasmodiophora brassicae* spores were observed from the resultant slurry. No symptoms or spores were present in any of the uninoculated plants. The pathogen is not a recent introduction to Maine. Pathogen buildup or spread to uninfested areas is a concern in Maine. Broccoli is susceptible to *Plasmodiophora brassicae* and is an important cash crop often used in rotation with potatoes in Maine. The economic impact to the broccoli industry has the potential to be greater than the impact to the canola industry. To our knowledge, this is the first report *Plasmodiophora brassicae* on spring canola in Maine.

White rot of garlic caused by *Sclerotium cepivorum* -- a new disease in Maine

S. B. JOHNSON (1), D. H. Lambert (1), B. Watt (1), R. Kersbergen (1)
(1) University of Maine, Orono, ME, USA
Phytopathology 100:S194

There are over 70 commercial garlic (*Allium sativum* var *ophioscorodon*) growers in Maine representing all 16 counties. Most garlic producers in Maine are market gardeners producing many crops. However, the contribution to farm income from garlic is disproportionably large when compared to the area planted. Garlic grown in Maine is distributed within Maine and to other states. In July of 2008, garlic plants were observed with symptoms which appeared to consistent with white rot. Severely affected plants were stunted with yellowing and wilting of the leaves. Bulb decay was present as were sclerotia. Infected plants were collected from the field. *Sclerotium cepivorum* Berk was isolated from the diseased bulbs. Established chives (*Allium schoenoprasum*) were used as susceptible host. These were inoculated with a sclerotia/mycelial mixture. Uninoculated chives plants were used as controls. All chives plants were examined twenty weeks after inoculation. Typical symptoms were evident in all of the inoculated plants. The pathogen, *Sclerotium cepivorum*, was isolated from the inoculated and symptomatic plants. No symptoms were present in any of the uninoculated plants. The pathogen is a recent introduction to Maine. While reputedly present a year or two previously, disease has now been confirmed. Pathogen buildup and spread to uninfested areas is a concern in Maine. The current practice of importation of seed stock and exchange of live plant material may contribute to new appearances and further spread of the disease. At present, there is no current knowledge on the distribution and prevalence of garlic white rot.

Solutions to the imbroglio over the nomenclature of *Gremmeniella abietina*

G. LAFLAMME (1)
(1) Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec, QC, Canada G1V 4C7
Phytopathology 100:S194

Scleroderris canker is caused by the fungus *Gremmeniella abietina*. In North America, the disease was first noticed in red pine plantations in Michigan, USA, in 1951 and was then referred to as the X disease. In 1954, similar damage was reported in Ontario, Canada. The causal agent was first identified in Maple, Ontario, in 1962, as *Scleroderris lagerbergii*. Taxonomists changed the name of the genus, in 1969, to *Ascocalyx* and *Gremmeniella*. Two years later, a third genus name appeared in the literature: *Lagerbergia*. Within the accepted species *Gremmeniella abietina*, three races were created in 1975: North American, European and Asian. In 1989, two varieties were recognized, one representing the three previous races and the second for *G. abietina* found on spruce and fir. In the 1990s, based on the host affected by the disease, a new vocabulary appeared: small tree type and large tree type, or, according to pathogen traits, types A and B. This was followed later by amplitypes (European, Northern, Alpine) which evolved later into biotypes (Alpine, Fennoscandian and European). A new biotype from Spain will soon be added to this list. Race and type are not valid ranks in fungal taxonomy. After molecular analyses, it now seems more evident that *G. abietina* is a European species, known in North America under the name European race. Fungi found on pine, fir and spruce in North America and on Todo-fir in Japan (the latter is

currently referred to as the Asian race) are four different species. The case of *G. laricina* being one or two species in Europe and North America remains to be clarified.

Integration of biofungicides and conventional fungicides for management of peach brown rot

N. LALANCETTE (1), K. McFarland (1)
(1) Rutgers University, Agricultural Research & Extension Center, Bridgeton, NJ, USA
Phytopathology 100:S195

The biorational fungicides Serenade MAX (*Bacillus subtilis* QST 713), Kaligreen (potassium bicarbonate), and Trilogy (hydrophobic extract of neem oil) were examined in integrated programs with conventional fungicides during the 2009 growing season for management of brown rot blossom blight and fruit rot on 'Encore' peach. Experimental programs consisted of low and high rates of each biofungicide applied during bloom (pink, full bloom, and petal fall timings) for blossom blight control and as the middle of three applications at 18-, 9-, and 1-day preharvest for rot control during the fruit ripening period. Treatments were applied using an airblast sprayer (935 L/ha) to single trees arranged in a randomized complete block design with four blocks; non-treated buffer trees surrounded each treatment tree. A standard commercial program and non-treated control (NTC) were included for comparison. Results of analyses of variance of both the blossom blight and fruit rot dependent variables showed significant model and treatment effects. Blossom blight canker incidences (% shoots with canker) for Trilogy-low (2.5%), Serenade-low (1.3%), Kaligreen-high (1.3%), and Trilogy-high (0%) were significantly less than the NTC (10.0%) and not significantly different from standard (0%). These integrated treatments provided 75–100% control of blossom blight canker development. At harvest, all six integrated programs had significantly less brown rotted fruit (22.0–35.3% fruit rot) than the NTC (90.4%) and statistically equivalent incidence of rot to the standard (16.2%). Similar results were obtained for a postharvest evaluation of fruit after 3-days incubation; integrated programs had 14.7 to 29.3% fruit with brown rot versus 26.3% for the standard. These results demonstrated that biorational fungicides can be integrated with conventional fungicides to provide effective brown rot control programs. Furthermore, the results suggest that early season blossom blight control may be possible with biofungicides alone. Data from additional seasons are required to substantiate these findings.

Evaluation of strawberry breeding lines for tolerance to black root rot and black vine weevil feeding

J. A. LAMONDIA (1), R. S. Cowles (1)
(1) Connecticut Agricultural Experiment Station, Windsor, CT, USA
Phytopathology 100:S195

Perennial strawberries are an important high value crop in the northeast U.S. Root diseases and root-feeding insects reduce yield and strawberry planting longevity. The most important root disease is strawberry black root rot, caused by *Rhizoctonia fragariae*. Feeding by root weevil larvae, especially black vine weevil (BVW), also reduces root mass and damages or kills plants. We conducted field assessment of strawberry cultivars over three years for yield, vigor, and root health to identify tolerance to black root rot as well as leaf feeding preference bioassays to identify tolerance to BVW. Several cultivars were identified as having characteristics desirable as parents for crosses. Primetime and Lester exhibited resistance or tolerance to black root rot and non-preference to BVW in feeding preference trials; the cultivar Idea was moderately susceptible to root rot, but produced a large, vigorous root system. Progeny of crosses made between Primetime, Lester, Allstar, Delmarvel, and Idea were carefully selected for resistance or tolerance to black root rot in greenhouse pots and in the field in infested soils as well as low preference in BVW feeding trials. Progeny were also screened for fruit yield, size, and flavor. Selection over three years reduced the progeny population from >4,000 genotypes to a few elite clones with promising horticultural characteristics, tolerance to black root rot, and low feeding preference by BVW. Our results demonstrated that sufficient variation exists in certain octoploid parents to develop effective resistant/tolerant lines. Because there were differences in disease reactions between greenhouse evaluations of juvenile plants and field evaluations on mature plants, evaluations in the field are essential in selecting for black root rot tolerance.

Changing climate, changing forests: Two decades of tree dieback and decline in Maine

W. H. LIVINGSTON (1)
(1) University of Maine, Orono, ME, USA
Phytopathology 100:S195

A number of tree dieback and decline episodes have occurred in Maine forests over the past 25 years: Ash dieback 1985–95 (*Fraxinus nigra*), island spruce decline 1995–2000 (*Picea glauca*), white pine decline 1995–2001 (*Pinus strobes*), beech decline 1999–2004 (*Fagus grandifolia*), and balsam fir decline 1999–2004 (*Abies balsamea*). In all studies, dendrochronology was the key analytical approach for establishing consistencies between likely inciting stresses and diebacks or declines. Historical abandonment of agricultural fields created conditions where native tree species regenerated on sites where they were not well adapted for long-term survival. In white pine decline, trees regenerated in high densities on sites where white pine rooting patterns would not penetrate deeply into the soil. A drought in 1995 incited a decline in these stands. On Maine coastal islands, abandonment of sheep pastures around 1900 allowed white spruce to regenerate in pure stands not historically found on the islands. As the stands matured, eastern dwarf mistletoe (*Arceuthobium pusillum*) and spruce beetle (*Dendroctonus rufipennis*) built-up populations and killed stands. Droughts are common inciting events in other diebacks and declines (ash, beech, balsam fir) in Maine where typical years do not have a dry season. Finally, warmer winter temperatures are allowing buildup of insect populations of beech scale (*Cryptococcus fagisuga*) and balsam woolly adelgid (*Adelges piceae*) that predispose trees to declines incited by drought. The combination of land use histories altering normal disturbance and successional patters, years of drought, warmer winters, and build-up of invasive pests have been adversely affecting survival of trees in Maine.

Sensitivity of *Phytophthora capsici* isolates from cucurbits in the northeastern U.S. to dimethomorph, cymoxanil, and mefenoxam

A. M. MADEIRAS (1), R. L. Wick (1), D. R. Cooley (1)
(1) University of Massachusetts, Amherst, MA, USA
Phytopathology 100:S195

Phytophthora capsici has become an important pathogen of cucurbit crops in the northeastern United States over the last few years. Monitoring fungicide resistance development in this pathogen is crucial to the success of disease control programs. The purpose of this study was to determine sensitivities of local *P. capsici* isolates to dimethomorph, cymoxanil, and mefenoxam. Single spore isolates from each of ten cucurbit fields in New York and Massachusetts were randomly selected and assayed. The 37 isolates tested for sensitivity to dimethomorph demonstrated a range of 50% effective concentration (EC50) values from 0.21 to 0.63 mg/L. Of 37 isolates tested for sensitivity to cymoxanil, 21 (56.8%) had EC50 values >50 mg/L and 16 (43.2%) had EC50 values <50 mg/L. EC50 values for cymoxanil ranged from 1.04 to 132.8 mg/L. Of 39 isolates tested, 35 (89.7%) were sensitive (relative colony diameter [RCD] <30% of nonamended control) and 4 (10.3%) were intermediately sensitive (RCD 30–90%) to mefenoxam. The results of this study parallel those of investigators in other locations and provide information which can be used to monitor changes in dimethomorph, cymoxanil, and mefenoxam sensitivity in populations of *P. capsici* on cucurbits in the northeastern U.S.

Elucidation and management of bacterial diseases on sweet onion in Pennsylvania

M. A. Mansfield (1), B. K. GUGINO (1)
(1) The Pennsylvania State University, University Park, PA, USA
Phytopathology 100:S195

Sweet onions are an emerging crop in Pennsylvania; however, they are susceptible to a number of bacterial diseases that cause leaf and bulb decay. In 2008, it was estimated that 50% of the sweet onion crop in Lancaster County was culled in the packing shed as a result of bacterial diseases. In addition, recent studies reported an association between disease incidence and severity on onions grown using plastic mulches typical of production in PA. Our objectives were to identify and characterize the bacteria associated with symptomatic onions in commercial fields and evaluate the effect of bare soil, straw and different types/colors of plastic mulches with and without thrips control on bacterial disease incidence and severity in a replicated field trial. Based on preliminary morphological identification and pathogenicity tests using an onion slice bioassay, *Burkholderia cepacia*, *B. gladioli*, *Erwinia caratovora*, *E. chrysanthemi*, *Pantoea ananatis*, *P. agglomerans*, *Pseudomonas syringae*, *P. viridiflora*, *Xanthomonas axonopodis*, and *X. campestris* were identified from symptomatic onions collected from 15 commercial fields across PA in 2009. This is the first report of *P. ananatis* and *P. agglomerans* causing disease on onion in PA. Further molecular characterization of these isolates is ongoing. In the field trial, bacterial diseases were most prevalent on onions grown on bare soil followed by those on white plastic mulch without thrips control. Additional data will be presented on the effects of mulch type and thrips control on onion bacterial disease incidence and severity and its implication for disease management.

Proposing a new species of *Fusarium*: *F.* 'aestuarinus,' a pathogen of *Spartina alterniflora* associated with wetland dieback in eastern marshes

R. E. MARRA (1), W. H. Elmer (1)
(1) Connecticut Agricultural Experiment Station, New Haven, CT, USA
Phytopathology 100:S195

Sudden Vegetation Dieback (SVD) is a syndrome characterized by rapid loss of vegetation, particularly smooth cordgrass (*Spartina alterniflora*). The

phenomenon has been observed in salt marshes of the eastern seaboard extending from Louisiana north to Maine. Morphological assessments of fungi associated with *S. alterniflora* in SVD sites have revealed a preponderance of isolates in the genus *Fusarium* that could not be assigned to known species. Based on morphology and greenhouse pathogenicity studies, the isolates separated into two groups, pathogens and nonpathogens. Phylogenetic analyses of three nuclear genes – beta-tubulin, calmodulin, and translation elongation factor 1-alpha – corroborated morphological and pathogenicity studies. Phylogenies were constructed using 20 pathogenic isolates, 18 nonpathogenic isolates, and nine previously described *Fusarium* species. Maximum Parsimony and Maximum Likelihood analyses, using individual-gene and combined-gene datasets, produced concordant topologies that strongly support the hypothesis that the pathogenic and nonpathogenic isolates constitute two phylogenetically distinct clades. From these data, we conclude that the pathogens represent a single species in the *Fusarium* section *Sporotrichiella*, for which we propose the name *Fusarium* 'aestuarinus.' Isolates in the nonpathogenic group further cluster into two distinct clades, both clearly belonging to the *F. incarnatum-equiseti* species complex. Additional analyses reveal that beta-tubulin sequences from *F. langsethiae* and *F. equiseti* share strongest similarities to that from a more distantly related ascomycete, *Arthrinium* sp., highly suggestive of horizontal gene transfer, and warranting further study.

Occurrence of basil downy mildew in the eastern U.S. in 2009

M. T. MCGRATH (3), C. A. Wyenandt (2), R. N. Raid (5), M. Babadoost (1), R. L. Wick (4)
(1) Department of Crop Sciences, University of Illinois, Urbana, IL, USA; (2) Department of Plant Biology and Plant Pathology, Rutgers University, Bridgeton, NJ, USA; (3) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, LIHREC, Riverhead, NY, USA; (4) Department of Plant Soil and Insect Sciences, University of Massachusetts, Amherst, MA, USA; (5) University of Florida, Everglades Research & Educ Ctr, Belle Glade, FL, USA
Phytopathology 100:S196

Downy mildew (caused by *Peronospora belbahrii*) is a new disease of basil in the USA. It was first detected in FL in Oct 2007. There were several reports in the eastern USA in 2008. Occurrence in 2009 was monitored through sentinel plots planted with the cucurbit downy mildew project plots throughout the eastern USA and a publicly-accessible spreadsheet on the web. Downy mildew was reported in 2009 on basil grown in greenhouses and outdoors, in both commercial crops and gardens. The first reported observation was 14 Jan in GA. Downy mildew was also reported in FL, SC, NC, TN, VA, DE, NJ, PA, NY, CT, MA, VT, IL, IN, ND, and CA. Not all reports were made by plant pathologists or confirmed by microscopic examination. Entries in the spreadsheet include the reporter's name and method of diagnosis. Downy mildew was reported widespread with large impact in some areas. In NJ, symptoms were first observed in June and progressed during the season throughout the production area which covers more than 600 A. Downy mildew was also widespread in FL, and in IL where commercial basil field production is estimated at 550 A. Entire crops have been lost because of this disease. Downy mildew is now recognized to be established in the USA and is anticipated to continue occurring every year. Until host plant resistance becomes available, it appears that downy mildew may force growers to make drastic changes in production practices, principally applying fungicides to a crop that rarely needed pesticide applications previously.

Control of foliar diseases in organically-produced tomato with biopesticides

M. T. MCGRATH (1), G. M. Fox (1)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University, LIHREC, Riverhead, NY, USA
Phytopathology 100:S196

Tomato is an important crop for organic vegetable growers. Foliar diseases occur commonly in the northeastern U.S. and can reduce fruit quality and quantity. EPA-defined biopesticides compliant with U.S. National Organic Program were evaluated in 2008 and 2009 for naturally-occurring diseases in trellised fresh-market tomato. Main diseases were powdery mildew and Septoria leaf spot. Applications were made weekly with a back-pack sprayer and hand-held boom beginning before symptoms were seen. In 2008, degree of control calculated from canopy severity on 6 Oct was 75% for powdery mildew and 58% for Septoria leaf spot with Actinovate SP (0.0371% *Streptomyces lydicus*). It was 98% and 68% with Companion (0.03% *Bacillus subtilis* GB03), 100% and 61% with Regalia (5% extract of *Reynoutria sachalinensis*), 99% and 54% with Organocide (5% sesame oil), and 86% and 0% for Sporatec AG (18% rosemary oil, 10% clove oil, and 10% thyme oil). Degree of control of both diseases obtained with Regalia alternated with Kocide 3000 (46% copper hydroxide), Organocide plus Kocide (both at low rates), and Sporatec plus Saf-T-Side was not significantly different from Kocide alone applied weekly or the conventional fungicide program (control of 99–100% and 94–100%). Copper fungicide is considered a standard being the main product used currently by organic growers. Another biopesticide, Taegro (24.5% *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24), was only effective for powdery mildew (56%). In 2009, Septoria leaf spot was the dominant disease. The most effective biopesticides were Companion and Actinovate. They were at least as effective as Kocide. Similar control was obtained with low rates of Organocide plus Kocide. The most effective treatments in 2009 included conventional fungicides used alone or combined with biopesticides.

Identification and characterization of silicon transporters in wheat (Triticum aestivum)

J. MONTPETIT (1), J. Vivancos (1), W. Rémus-Borel (1), C. Grégoire (1), F. Belzile (1), R. R. Bélanger (1)
(1) Université Laval, Quebec, Quebec, Canada
Phytopathology 100:S196

Silicon (Si) is not considered as an essential element for plant growth but its supply is known to be beneficial, namely in preventing biotic and abiotic stresses. However, its positive effects are variable since in planta accumulation differs among plant species and a direct correlation between benefits and absorption has been shown. Some species of the *Gramineae* family can accumulate up to 10% on a dry weight basis while others only accumulate less than 0.1%. Recently, studies with rice have shown that Si transport is mainly regulated by two genes, *Lsi1* and *Lsi2*. *Lsi1-2 like* genes have also been reported in barley and corn. The objective of this project was to identify and characterize orthologous Si-transport genes in wheat, given the ability of this plant to accumulate high Si concentrations. With the design of degenerate primers, two genes presenting high homology (>80%) to *Lsi1* and *Lsi2* in rice were identified. Following their isolation, the Si-transport activity of *Lsi1* was verified in *Xenopus laevis* oocytes, a heterologous system. Functional characterization is in progress in the model plant *Nicotiana tabacum* by the intra-cellular localization of these transporters. Preliminary results suggest that *Lsi1* transporter in wheat is localized across the entire plasma membrane, unlike *OsLsi1* located in specific distal parts of the cell. This localization of Si transporters could explain the difference in Si absorption between wheat and rice.

Breeding hybrid poplar in Québec to improve their resistance to *Septoria musiva*

M.-J. MOTTET (1), P. Périnet (1)
(1) Ministère des Ressources naturelles et de la Faune, Direction de la recherche forestière, 2700, rue Einstein, Québec, Qué., Canada G1P 3W8
Phytopathology 100:S196

The poplar (*Populus* spp.) breeding program in Québec is based on multi-trait selection including disease resistance. Infection by the native fungus *Septoria musiva* Peck causes severe stem deformation and breakage leading to top dieback or death of the trees. In Québec, bioclimatic conditions have a significant impact on canker incidence and severity; damage generally decreases following a south-north gradient. For about 20 years the breeders have been selecting clones to improve Septoria canker resistance. Interspecific hybridization aims to capture growth vigour, cold hardiness, and site adaptability from the species *P. trichocarpa* (T) and *P. maximowiczii* (M) while retaining Septoria resistance from *P. deltoides* (D), and to some extent *P. nigra* (N) and *P. balsamifera* (B). Despite the high susceptibility of T and M species and considering that Septoria resistance is apparently recessive, improvement in resistance was accomplished in some species and hybrids (T, N, TD, DN x M, NM, MB) through selection and testing. The objective is to incorporate resistance or tolerance genes originating from many sources in order to achieve durable resistance. Progenies or clonal populations are first screened in nursery or early tests where artificial inoculations with different isolates contribute to accelerate the screening. Then, superior clones are tested in several locations including Septoria prevalent sites. More than 5000 clones have been evaluated since 1969. For now, about 40 clones belonging to different hybrid types and families are used for planting in Québec including 18 resistant/tolerant clones in Septoria zones. Some of those clones are periodically replaced to improve traits of interest and increase genetic diversity. Even if Septoria canker expands in geographic range since year 2000, selection for Septoria resistance has not been defeated by evolution of new races or other pest.

The potential for post-harvest foliar urea sprays to reduce ascospore production by *Venturia inaequalis*

R. NORTON (1), C. A. Smith (1), W. E. MacHardy (1), W. Lord (1)
(1) University of New Hampshire, Durham, NH, USA
Phytopathology 100:S196

Apple scab, a disease caused by the ascomycete *Venturia inaequalis,* is an important disease of apples in the Northeast. In the Northeast, apple scab is

typically controlled by frequent applications of chemical fungicides in the spring. Consumer concerns about the potential harmful effects of pesticides on human health and the environment has created a need for alternative management options. We are examining the use of urea, as an alternative to chemical fungicides for controlling apple scab. Although previous studies have shown that urea reduces the production of ascospores, the primary inoculum for apple scab, the studies did not compare application rate, timing of application, and winter hardiness in a single comprehensive study. Cortland leaves, infected with *V. inaequalis,* were treated immediately post-harvest, at the start of leaf fall, and at 95% leaf fall. A 5% urea application was made either as a single spray or in split applications. Our data suggests that post-harvest foliar urea applications significantly reduce the production of ascospores in *V. inaequalis.* In conjunction with the ascospore study, we are also examining the effects of post-harvest foliar urea sprays on winter hardiness, fruit set, and foliar nitrogen content. Results indicate that post-harvest foliar urea applications have no adverse effects on tree health.

The effect of demethylation inhibitor fungicides on *Sclerotinia homoeocarpa* population structures

J. T. POPKO (2), C. Ok (2), W. R. Autio (2), M. T. McGrath (1), G. Jung (2)
(1) Department of Plant Pathology and Plant-Microbe Biology, Cornell University; (2) Department of Plant, Soil and Insect Sciences, University of Massachusetts-Amherst
Phytopathology 100:S197

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is the most economically important turfgrass disease in North America. Dollar spot is primarily controlled by fungicide application on golf courses; however, fungicide resistance has been confirmed in three of the five fungicide classes used to control dollar spot. Among the confirmed classes, the sterol demethylation inhibitor (DMI) fungicide class is the most widely used. The objective of this project was to investigate the effect of propiconazole (DMI) rates on changes in dollar spot population structure using in-vitro fungicide assays and field efficacy results. Two sites (Hickory Ridge Country Club, HRCC and South Deerfield Turf Research Center, SDTRC) were selected for the experiment. Dollar spot was sampled prior to fungicide application and at the end of the experiment to examine change in population structure. Samples were also taken 7 and 14 days after fungicide application from infection centers that displayed actively growing mycelia to determine the sensitivity of isolates causing reduced DMI efficacy. All samples were subjected to an in vitro fungicide assay using a single discriminatory concentration (0.1 µg a.i./ml) of propiconazole to determine relative mycelia growth percentage (RMG%). Propiconazole (0.44, 0.88, 1.28 and 1.72 kg a.i. ha^{-1}) and the industry standard chlorothalonil (non-DMI/12.67 kg a.i. ha^{-1}) fungicides were applied to both sites to test field efficacy. The initial sampling from the HRCC (n = 433 isolates) revealed the pre-existence of DMI sensitive and insensitive sub-populations. All isolates from SDRC (n = 458) were DMI sensitive. All samples from propiconazole treated plots 7 and 14 days after application contained only isolates from the insensitive sub-population regardless of rate applied at HRCC. Non-DMI treated plots (untreated or chlorothalonil) sampled 7 and 14 days after application contained isolates of both sub-populations. Reduced field efficacy using propiconazole was observed at HRCC, whereas complete control was observed at the SDRC.

Structural defense mechanisms in trees: What's new?

D. RIOUX (1)
(1) Natural Resources Canada
Phytopathology 100:S197

As is the case for many herbaceous plants, preexisting defense structures are essential in helping trees resist pathogens or mechanical damage. For instance, by insulating the inner living tissues from heat damage, the thick bark of sequoia trees is considered an important factor in their tolerance to fire. Another example is the wax that covers the foliage that often has an influence on the germinating rate of some pathogen spores or can even help prevent the penetration of stomata. When such preformed defense elements fail to impede pathogen ingress, trees respond by forming different structures to limit or stop pathogen invasion. Compartmentalization processes are certainly among the most important induced mechanisms that explain tree resistance to various stresses. Basically, it involves the formation of barriers that bound infected tissues and thus limit the extent of such lesions in trees. Lignin and suberin often impregnate the walls of cells involved in compartmentalization whereas phenols are usually a major component of their cytoplasm. Lately, resistance of eucalyptus trees to a leaf pathogen has been attributed to these types of compartmentalization responses. Likewise, in a recent study, it was clearly shown that such reactions occur in elm calli inoculated with a wilt pathogen. Genes and substances potentially involved in metabolic pathways leading to compartmentalization barriers have been reported in recent years. In particular, methyl jasmonate can induce the formation of traumatic resin canals in conifers, and these canals are regularly found in compartmentalization xylem barriers. Interestingly, embolism seems to be a significant trigger of compartmentalization and this could result in practical applications, e.g. when sugar maple trees are tapped to collect their sweet sap. Finally, even though compartmentalization structures are composed of antifungal compounds, some fungi have developed strategies to breach these defensive tissues.

Summer N-fertilization effects on annual bluegrass putting green turf

J. A. ROBERTS (1), J. A. Murphy (1), B. B. Clarke (1)
(1) Rutgers University, New Brunswick, NJ, USA
Phytopathology 100:S197

Anthracnose, caused by *Colletotrichum cereale* Manns, is a devastating disease of putting green turf. Increased N fertility has been reported to reduce anthracnose severity on annual bluegrass [*Poa annua* L. f. reptans (Hausskn) T. Koyama] turf. In 2007, a 3-yr field study was initiated in North Brunswick, NJ to determine the effect of rate and frequency of soluble-N fertility during mid-season on anthracnose severity of annual bluegrass turf maintained at 3.2 mm. The date of initiating N fertilization (mid-May vs. mid-June) was also evaluated during 2008 and 2009. Nitrogen was applied at 4.9 kg ha^{-1} every 1, 2, 4 and 8 wk and 9.8 kg ha^{-1} every 2 and 4 wk as a solution of NH_4NO_3. Anthracnose severity, assessed as area under the disease progress curve, was reduced linearly with increasing total N rate (9.8 to 58.8 kg ha^{-1}). Nitrogen applied at 58.8 kg ha^{-1} total (4.9 kg ha^{-1} wk^{-1} or 9.8 kg ha^{-1} 2 wk^{-1}) had the greatest reduction in anthracnose severity throughout the study. Nitrogen applied at 29.9 kg ha^{-1} over the season (4.9 kg ha^{-1} 2 wk^{-1}) was the lowest N rate to significantly reduce disease severity, and anthracnose was most severe on turf receiving N at 19.6 and 9.8 kg ha^{-1} (4.9 kg ha^{-1} 4 wk^{-1} or 8 wk^{-1}) over the season. Initiating N fertilization before symptom expression (mid-May) reduced anthracnose severity compared to fertilization initiated at the onset of disease (mid-June) in 2008. Thus, fertilization techniques that increased mid-season N fertility, within the range of 29.9 to 58.8 kg ha^{-1}, were effective at reducing anthracnose severity on annual bluegrass turf.

Using lime-sulfur to control sooty blotch and flyspeck in organic apple production in southeastern New York State

D. A. ROSENBERGER (1), F. W. Meyer (1), A. L. Rugh (1)
(1) Cornell University's Hudson Valley Lab, Highland, NY, USA
Phytopathology 100:S197

Lime-sulfur (LS) was applied to control sooty blotch and flyspeck (SBFS) on apple fruit in five trials in which replicated plots were sprayed to drip using a handgun. In 2005, Golden Delicious apples receiving two sprays of LS at 10 ml/L during July followed by one spray of thiophanate-methyl plus captan (210 and 600 mg/L of active ingredient) in Aug had no more SBFS than trees that received thiophanate-methyl plus captan (TM-C) in all three sprays. In 2006, four summer applications of LS at either 5 or 10 ml/L on a 20-day interval or six sprays at 2.5 ml/L on a 10-day interval controlled flyspeck just as well as four sprays of TM-C, but a four-spray program of LS at 2.5 ml/L was less effective. In 2007, LS at 2.5 ml/L was applied alone on 7 and 30 June and in a tank mix with 300 mg/L of Cuprofix Disperss (71% basic copper sulfate) on 24 July and 14 Aug. This treatment reduced flyspeck by only 77–78% on Empire and Golden Delicious fruit compared to unsprayed controls whereas TM-C provided 94–96% control. During the very wet summer of 2009, 98% of unsprayed Royal Court fruit failed to meet the U.S. Fancy grade at harvest due to SBFS whereas eight summer sprays of LS at 2.5 ml/L resulted in 65% out-of-grade fruit. When the eight-spray LS program was modified by applying LS alone in June and Sep but adding 318 mg/L of Nordox (56% copper oxide) to LS in the four July-Aug sprays, only 32% of fruit were down-graded for SBFS but 26% showed copper injury. LS can be used to control SBFS in organic apple production, but additional research is needed to optimize rates and spray timings and to determine if LS applied during summer reduces fruit size.

Stem susceptibility of six eastern Canadian tree species to *Phytophthora ramorum*

M. SIMARD (2), S. C. Brière (1), A. K. Watson (3), D. Rioux (2)
(1) Canadian Food Inspection Agency; (2) Canadian Forest Service; (3) McGill University
Phytopathology 100:S197

Phytophthora ramorum (Pr) is an emerging pathogen that causes diseases known as sudden oak death, ramorum leaf blight and ramorum shoot dieback. Even though Pr has been reported to naturally infect around 120 species, it has not been detected in the wild in eastern North America. However, there is real concern that Pr could be introduced and spread into this area. To better estimate this risk, seedlings of the following six eastern Canadian forest species were stem-inoculated with Pr: *Abies balsamea* (Ab), *Acer saccharum* (As), *Betula alleghaniensis* (Ba), *Fraxinus americana* (Fa), *Larix laricina* (Ll), and *Quercus rubra* (Qr). Bark necrosis, colonization by Pr as well as host defense reactions were evaluated. Two months after inoculation, nearly 25%

of Ll and Ab seedlings died. Necrotic areas on the bark were larger in Ll, Ab, and Qr than in Fa, As, and Ba. Chlamydospores were observed close to the inoculation point in the phloem of Ll, Ab, and Qr. Pr hyphae were abundant in the phloem and cambium but also in the xylem of the two coniferous species where the colonization is facilitated mainly through the invasion of ray cells. In broadleaf species, hyphae were observed in a few xylem vessels and fibers close to the inoculation point, except for Qr where Pr was abundant in xylem vessels and still present up to 5 cm above the inoculation wound. However, among the six species, Qr was the only one where defense reactions were clearly apparent, especially when the inoculation occurred later in the growing season. Overall, Ab, Ll, and to a certain extent Qr appeared susceptible to Pr and they could be killed should Pr be introduced during conditions conducive to disease development.

Molecular characterization of the biocontrol activity of *Pseudozyma flocculosa*

B. TEICHMANN (1), F. Lefebvre (1), C. Labbé (1), R. Bélanger (1)
(1) Biocontrol Laboratory, Pavillon de l'Envirotron, Université Laval, 2480, boul Hochelaga, Québec, G1V 0A6, Canada
Phytopathology 100:S198

The basidiomycetous fungus *P. flocculosa* is a natural inhabitant of the phyllosphere and has been isolated as a biocontrol agent (BCA) against powdery mildews. It secretes large amounts of an antifungal cellobiose lipid, flocculosin, presumably involved in its biocontrol activity. However, the molecular and genetic basis of glycolipid production and secretion is largely unknown in *P. flocculosa*. The related fungus *Ustilago maydis* secretes a highly similar glycolipid, ustilagic acid (UA), which also displays antibacterial and antifungal activity. Recently, a biosynthetic gene cluster was characterized in U. maydis and found to contain all genes required for the efficient production and secretion of UA. By analyzing the database of the recently sequenced genome of *P. flocculosa*, we hypothesized that a homologous gene cluster regulating flocculosin synthesis could be found in *P. flocculosa*. Comparison of the sequences of all 12 genes against the genome of *P. flocculosa* revealed that they were also present within a specific cluster with the exception of one gene encoding the alpha-hydroxylase Ahd1, necessary for alpha-hydroxylation of the fatty acid. On the other hand, the flocculosin gene cluster contained an additional gene encoding an acetyl-transferase, probably involved in the acetylation of a further acetyl-group at the cellobiose moiety. It has already been shown that the presence of powdery mildew on a plant leaf triggers strong growth of *P. flocculosa* thereby affecting the pathogen. It remains to be elucidated which role flocculosin plays in this biocontrol activity. One hypothesis is that the release of flocculosin leads to formation of lesions in the membrane of the pathogen cells followed by the release of nutrients stimulating growth of the BCA. To validate this hypothesis, we are currently trying to generate a mutant strain deficient in its ability to produce flocculosin in order to analyze the biocontrol potential of the resulting phenotype.

Evaluation of basil (*Ocimum* spp.) cultivars and breeding lines for susceptibility to downy mildew

A. WYENANDT (2), J. Simon (1), C. Park (3)
(1) Director, New Use Agriculture and Natural Plant Products Program (NUANPP), Department of Plant Biology and Plant Pathology, Rutgers University, New Brunswick, NJ, USA; (2) Extension Specialist in Vegetable Pathology, Department of Plant Biology and Plant Pathology, Rutgers University, Bridgeton, NJ, USA; (3) Research Associate, New Use Agriculture and Natural Plant Products Program (NUANPP), Department of Plant Biology and Plant Pathology, Rutgers University, New Brunswick, NJ, USA
Phytopathology 100:S198

Since 2007, downy mildew (*Peronospora belbahrii*) on sweet basil (*Ocimum basilicum*) has caused significant losses in New Jersey and other basil production areas of the eastern U.S. No known resistance in basil to downy mildew has been reported. In 2008, different basil species, cultivars, and Rutgers University breeding lines (30 in total) were evaluated for susceptibility to downy mildew in a field trial in southern New Jersey. On 27 Jul, all basil was hand transplanted in a randomized complete block design with four replications. The field was artificially-infested with downy mildew by transplanting infected sweet basil plants into rows on 31 Jul. On 20 Aug and 21 Sep basil plants were rated for downy mildew infection using a plus scale rating system. *Ocimum basilicum* was the most susceptible among all *O.* species and varieties evaluated. While sporulation ratings varied among the sweet basil varieties, popular commercial cultivars such as 'Martina', 'Nufar' and 'Poppy Joe's' were among the most susceptible. Symptoms and sporulation on *Ocimum* × *citriodorum* and *O. americanum* cultivars were present, but far less than on *O. basilicum* cultivars. 'Spice', 'Blue Spice', and 'Blue Spice Fil' were the least susceptible to basil downy mildew with no visible symptoms developing on the leaves. Similar findings were observed on a second but non-inoculated basil cultivar trial in northern New Jersey. This is the first report of potential resistance in *Ocimum* spp. to downy mildew. Observations from this study show that genetic resistance is possible. Selection criteria such as foliar morphology, plant architecture, as well as, the presence of secondary metabolites are being examined as potential avenues for developing downy mildew resistance basil cultivars.

Wetwood – An ignored corner in forest pathology

D. YANG (1)
(1) FPInnovations-Forintek Division, 319 rue Franquet, Quebec, QC, Canada, G1P 4R4
Phytopathology 100:S198

Wetwood, or water pocket, is caused by anaerobic bacteria and occurs in many softwood and hardwood species. The bacteria enter trees through wounds or roots and produce pectinolytic enzymes to destroy the vessel and ray pit membranes of wood. The reproduction and metabolites of these bacteria form a foetid liquid in wood, which results in a high moisture content (MC) of the wetwood. Because the MC of wetwood is much higher than average, wetwood usually requires relatively long periods for adequate drying in sawmill. Degradation of pectic substances of the middle lamella causes weakness in the chemical bonds between wood cells. Consequently, weak bonding increases the risk of warping and checking in lumber during drying process. Wetwood also has a lower permeability than normal wood; this, in turn, affects the wood's treatability with preservatives. The economic losses resulting from wetwood for wood production and utilization are enormous. Many studies have been conducted in sawmill on drying wetwood using various physical, chemical, biological or mechanical methods, but the problem has yet to be solved. Studies on wetwood infection mechanisms and its control measurements in forest are limited. More attention should be given to wetwood problem, and wetwood-free trees are required for lumber manufacturing and wood utilization.

2010 Southern Division Meeting Abstracts

Abstracts presented at the APS Southern Division meeting in Orlando, Florida, February 7–8, 2010. The abstracts are arranged alphabetically, by first author's name.

Implications of fungicide application timings and irrigation on disease control and peanut yield

J. AUGUSTO (1), T. Brenneman (1)
(1) Dept. Plant Pathology, UGA, Tifton, GA, USA
Phytopathology 100:S199

Night application and/or fungicide redistribution with irrigation may improve control of stem rot (*Sclerotium rolfsii*) and increase peanut (*Arachis hypogaea* L.) yield by enhancing fungicide penetration to the lower canopy. Four applications of chlorothalonil (1.26 kg a.i./ha), prothioconazole plus tebuconazole (0.23 kg a.i./ha), tebuconazole (0.21 kg a.i./ha), flutolanil plus propiconazole (0.45 kg a.i./ha) or pyraclostrobin (0.21 kg a.i./ha), and two applications of fluoxastrobin (0.17 kg a.i./ha) or azoxystrobin (0.31 kg a.i./ha) were applied either early morning (AM = 3 - 5 a.m., with folded leaves) or during daylight (PM = 10 a.m. - 12 p.m., with unfolded leaves) in irrigated and nonirrigated plots to evaluate disease control and peanut yield in 2008 (dry year) and 2009 (wet year). In 2008 leaf spot control was similar regardless of spray timings, fungicides, or irrigation. The AM application of all systemic fungicides except fluoxastrobin decreased stem rot in nonirrigated plots, but only azoxystrobin and prothioconazole plus tebuconazole decreased stem rot more in AM than in PM sprays in irrigated plots. Yields were higher with AM sprays of tebuconazole and prothioconazole plus tebuconazole in nonirrigated plots, and with flutolanil plus propiconazole, pyraclostrobin, tebuconazole and prothioconazole plus tebuconazole in irrigated plots than with PM sprays. In 2009, leaf spot was severe and spray timings with systemic fungicides gave similar control regardless of irrigation; pyraclostrobin had the lowest ratings. The AM sprays of pyraclostrobin, flutolanil plus propiconazole and prothioconazole plus tebuconazole had lower stem rot and higher yields than PM sprays, irrespective of irrigation. The effects of spray timings and irrigation on fungicide efficacy are not the same for all products.

Bacterial leaf scorch of blueberries: A new threat to the southeastern industry

P. M. BRANNEN (2), L. Nissen (2), T. Denny (2), C. Chang (3), M. Tertuliano (1)
(1) Entomology Dept., Univ. of Georgia, Tifton, GA; (2) Plant Pathology Dept., Univ. of Georgia, Athens, GA; (3) Plant Pathology Dept., Univ. of Georgia, Griffin, GA
Phytopathology 100:S199

The *Xylella fastidiosa* bacterium is the causal agent of bacterial leaf scorch (BLS) of blueberry, predominantly a problem on southern highbush cultivars (*Vaccinium corymbosum* interspecific hybrids), but also more recently confirmed to be present in rabbiteye (*Vaccinium virgatum*) cultivars. Symptoms include marginal leaf scorch, leaf drop, yellowing of stems, and eventual plant mortality. Initial typing of the blueberry strain places it in an A-type category, not closely related to Pierce's disease (G-type) strains. The glassy-winged sharpshooter, *Homalodisca vitripennis*, a known insect vector of other *X. fastidiosa* diseases, has been established as a potential vector of the bacterium in Georgia, since it is the most prevalent sharpshooter found in commercial blueberry plantings. A 2008 survey determined the prevalence of BLS in Georgia, and 71.1% of farms were positive for BLS in at least one field. Field resistance or tolerance was observed among some cultivars. However, highly susceptible cultivars are predicted to incur complete loss within 10 years of planting.

Efficacy of fungicides applied in furrow for peanut disease control

T. B. BRENNEMAN (1), J. Augusto (1)
(1) Department of Plant Pathology, University of Georgia, Tifton, GA
Phytopathology 100:S199

Prothioconazole (0.20 kg/ha), azoxystrobin (0.11 kg/ha), or penthiopyrad (0.35 kg/ha) were applied to Tifguard peanut (*Arachis hypogaea*) in furrow (spray volume 35 L/ha) at planting in two replicated trials in 2009. All plots received only chlorothalonil during the season for foliar diseases. Data for the trials could be combined, and none of the treatments affected plant stands. Expanding leaves were bioassayed with *Sclerotium rolfsii* after emergence but no residues were detectable. However, prothioconazole reduced leaf spot at harvest whereas other treatments did not. Prothioconazole and penthiopyrad each reduced stem rot at both midseason and harvest, but only penthiopyrad significantly increased yield (512 kg/ha greater than the nontreated control). In furrow fungicides are known to reduce diseases of seedlings and roots, but can also reduce foliar diseases of peanut.

Evaluation of *Pasteuria usgae* as a biological control of sting nematode (*Belonalaimus longicaudatus*)

J. H. CAMPBELL (1), J. L. Starr (1), K. L. Ong (1)
(1) Dept. of Plant Pathology & Microbiology, Texas A&M University, College Station, TX, USA
Phytopathology 100:S199

The plant-parasitic sting nematode (*Belonalaimus longicaudatus*) is one of the most devastating nematode pests on turfgrass. In recent years, the turfgrass industry has seen a number of chemical control measures taken off the market leaving no effective alternative. The effectiveness of a commercial formulation of *Econem* (*Pasteuria usgae*, an obligate bacterial parasite specific to sting nematodes) was tested on a bermudagrass putting green in Texas. A complete-block design with five replicates of each treatment was used. Granular applications of 100 thousand (30 g product formulation) or 200 thousand spores (60 g product formulation) per 16 square foot plot were applied monthly from April through July 2009. Effects of treatments on nematode population densities, root health and length, turf color and turf density were evaluated over time. There was no effect of treatments on sting nematode populations, root health or turf density. Turf color was significantly greater at both the 100k and 200k levels from the untreated controls at the 0.05 level. Average root length was statically greater at the 200k level than the other treatments. Less than two percent of nematodes in bacterial treated plots were encumbered by *P. usgae* endospores at the termination of the experiment.

Components of resistance to *Cercospora arachidicola* in medium maturity peanut varieties with moderate early leaf spot resistance in the field

E. G. CANTONWINE (2), A. K. Culbreath (1), D. Baskin (2), D. Murphy (2)
(1) University of Georgia, Tifton, GA, USA; (2) Valdosta State University, Valdosta, GA, USA
Phytopathology 100:S200

Until recently, resistance to early leaf spot, caused by *Cercospora arachidicola*, has been linked to late-maturity in peanut (*Arachis hypogaea*). An experiment was conducted to evaluate the components of resistance to *C. arachidicola* in two medium maturity peanut cultivars with moderate field resistance, Georgia-03L and Tifguard. Their responses to inoculation were compared to those of Georgia Green, a susceptible medium maturity cultivar, and two resistant late-maturing cultivars, Georganic and York. Leaves taken from the second position of flowering plants were detached, placed in beakers of saturated sand, and inoculated with spores of *C. arachidicola*. Leaves were maintained in a dew chamber at 24°C, 100% relative humidity, and 12-hr photoperiod for 32 days. Infection frequency, lesion diameter, incubation period, latent period, and the number of spores per lesion area were compared for the genotypes. The only resistance component observed for Georgia-03L was a reduced infection frequency, 0.35 lesions/cm compared to 0.53 lesions/cm for Georgia Green. Tifguard had a lower infection frequency (0.29 lesions/cm), smaller lesion diameter, longer latent period, and fewer spores per lesion area than Georgia Green. Infection frequency was also lower for York (0.23 lesions/cm) and Georganic (0.40 lesions/cm) than Georgia Green, and York and Georganic had smaller lesions than Georgia Green. York had fewer spores per lesion area than all cultivars tested.

Effect of cotton cultivar selection on soil populations of *Fusarium oxysporum* f. sp. *vasinfectum*

S. CHAWLA (1), J. E. Woodward (3), T. Wheeler (2)
(1) Department of Plant and Soil Science, Texas Tech University, Lubbock, TX, USA; (2) Texas AgriLife Research, Texas A&M System, Lubbock, TX, USA; (3) Texas Tech University, Lubbock, TX, USA & Texas AgriLife Extension Service, Texas A&M System, Lubbock, TX, USA
Phytopathology 100:S200

Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), is an important disease of cotton (*Gossypium hirsutum* L.) in portions of West Texas. A microplot study was conducted over the 2008 and 2009 growing seasons to investigate the influence of planting susceptible and/or resistant cotton cultivars, FiberMax (FM) 9058F and Stoneville (ST) 4554B2F, respectively on soil population of *Fov*. Fibermax cultivars, when planted 2 consecutive years resulted in large increase of Fusarium wilt. The hypothesis was that cultivars can affect population density of *Fov* in the soil. Microplots (75 cm diameter × 45 cm deep) were augmented with field soil naturally infested with *Fov* and *Meloidogyne incognita*. Treatments consisting of rotation schemes containing ST 4554B2F and FM 9058F were arranged in a randomized complete block with nine replications. Baseline soil populations (46.2 cfu/g soil) were enumerated for each microplot via soil dilution plating on a semi-selective medium. It was observed that FM 9058F planted in sequential seasons increased Fov populations (79.4 cfu/g soil); however, populations in microplots planted to ST 4554B2F over two seasons remained constant (45.8 cfu/g soil). Soil populations in microplots initially planted to FM 9058F followed by ST 4554B2F remained unchanged (44.2 cfu/g soil); whereas, *Fov* populations in microplots initially planted with ST 4554B2F followed by FM 9058F were not different from those planted to FM 9058F for two seasons. Results from this study may be useful in developing long term management strategies that can be implemented into integrated programs for sustaining the production of cotton in fields infested with *Fov*.

Effects of chlorothalonil and dodine applied alone and in combination with systemic fungicides on late leaf spot of peanut

A. K. CULBREATH (1), T. B. Brenneman (1), R. C. Kemerait (1)
(1) University of Georgia, Tifton, GA
Phytopathology 100:S200

In the southeastern U.S., control of late leaf spot, caused by *Cercosporidium personatum*, of peanut (*Arachis hypogaea*) requires multiple applications of fungicides. Recent renewed interest in the fungicide dodine for leaf spot control prompted comparison of this fungicide to chlorothalonil alone and in combination with four systemic fungicides in a randomized complete block field experiment at Tifton, GA in 2009. All fungicides were applied seven times at ca.14 day intervals starting at 37 days after planting. Leaf spot epidemics were severe, with final leaf spot severity ratings (Florida 1-10 scale) of 9.3 (> 95% defoliation) for the nontreated control. The dodine (0.45 kg a.i./ha) treatment had final leaf spot severity ratings of 8.1 compared to 5.1 for 1.26 kg a.i./ha of chlorothalonil (LSD = 0.7). Final leaf spot ratings for mixtures of dodine (0.3 kg a.i./ha) with propiconazole (0.063 kg a.i./ha), thiophanate methyl (0.2 kg a.i./ha), tetraconazole (0.063 kg a.i./ha) and cyproconazole (0.03 kg a.i./ha) were 7.2, 7.5, 6.1 and 6.6, respectively, compared to 4.8, 5.0, 5.1, and 5.2 (LSD = 0.7) for chlorothalonil (0.84 kg a.i./ha) for those respective treatments.

Comparative evaluation of the survivability of *Acidovorax avenae* subsp. *citrulli* in stored seeds

B. DUTTA (1), R. Walcott (1)
(1) University of Georgia, Athens, GA, USA
Phytopathology 100:S200

Bacterial fruit blotch (BFB), one of the most economically important bacterial diseases of cucurbits worldwide, is caused by *Acidovorax avenae* subsp. *citrulli* (*Aac*). Infested seeds are the primary source of inoculum and under favorable environmental conditions, up to 100% yield loss can occur. Recent reports indicating that *Aac* can be transmitted to seedlings from infested seeds stored for more than 38 years, suggest that the bacterium can withstand desiccation during storage. However, no detailed studies have been conducted to dissect the mechanisms of long term bacterial survival in seeds. Hence, the objective of this work was to compare the ability of *Aac* to survive on host and non-host seeds with *Xanthomonas campestris* pv. *campestris* (*Xcc*), *Pantoea stewartii* subsp. *stewartii* (*Pnss*) and *Ralstonia solanacearum* (*Rs*). Watermelon, tomato, cabbage, and corn seeds (n = 100 g) were artificially inoculated (separately) with suspensions containing 10^8 CFU/ml of each of the four bacteria. Inoculated seeds were air-dried overnight and stored at 4°C and 50% R.H. The bacterial populations on five replicated samples (n = 1 g of seed) from each treatment were estimated weekly for 3 months on semi-selective media. In two independent trials, the *Pnss* was undetectable on all seed types at the end of 3 months. In contrast, the populations of *Aac* and *Xcc* declined to 10^2 to 10^3 CFU/g of seeds whereas *Rs* populations declined to 10^4 to 10^5 CFU/g of seeds, irrespective of seed type. Seed type was not a significant factor in bacterial survival. These data suggest that *Aac*, *Xcc*, and *Rs* are more tolerant to desiccation than *Pnss*. The data also suggest that it is likely that the ability of *Aac* to survive for 38 yrs in stored seed is due to the location of the bacterium in the seed rather than some unique characteristic of the bacterium.

Current status and future of HLB

T. GOTTWALD (1), M. Irey (2), A. Bergamin-Filho (3), R. Bassanezi (4)
(1) USDA-ARS, Fort Pierce, FL; (2) Southern Gardens Citrus, US Sugar Corp., Clewiston, FL; (3) ESALQ, Universidade de São Paulo, Brazil; (4) Fundecitrus, Araraquara, Brazil
Phytopathology 100:S200

Results from studies on the increase in HLB incidence and spread in China and Reunion Island indicate a rate of disease increase leading to a multi-year epidemic requiring 7 to 10 years for infection to approach an asymptote of 100%. In contrast, more recent studies in Brazil, Vietnam, and Florida suggest a much more rapid rate of disease increase and spread. An HLB epidemic was examined in a plantation of over 4,800 ha in South Florida where no new citrus had been introduced for 10 y and thus spread was entirely dependent on psyllid transmission. The level of psyllid infestation was unprecedented compared to previously recorded psyllid infestations. The psyllid vector was relatively newly introduced to Florida and thus lacks the biological and environmental constraints found in its native range. Consequently the HLB epidemic in Florida is undoubtedly one of the worst on record. Stochastic Markov-Chain Monte Carlo models indicated a prevalence of secondary spread with occasional primary spread from outside the plots. Interpretations of the stochastic models combined with survival analyses show spread over multiple scales from local to regional are occurring simultaneously and continually in Florida. Edge effects analyses indicate a prevalence of infections that accumulate at the transition of plantings and areas devoid of citrus such as the plantation perimeter, internal roads, canals, ponds, etc. This edge effect diminishes rapidly toward the interior of the planting and is generally well described by an inverse power function.

MeloCon WG® and SoilGard 12G® used in a program as a methyl bromide alternative to control nematodes and soil borne diseases in fruiting vegetables

H. B. HIGHLAND (1)
(1) Certis USA, Nokomis, FL
Phytopathology 100:S200

With the advent of the Montreal Accord of 2007 on restricting ozone depleting gases, and as a result of further state led restrictions, the use of methyl bromide and other fumigants in agriculture has been on a steady decline. As such effective and safe alternative treatments are being investigated, labeled and used in commercial production. The loss of fumigants is especially deleterious to the production of fruiting vegetables, primarily tomatoes and peppers, in the southeastern US, where soil borne diseases and nematodes can be of particular concern. A program of MeloCon® WG and SoilGard® 12 G, marketed by Certis USA, have been shown to be very effective when used alone or in combination to control nematodes and

soil pathogens in field trials in the US. MeloCon® WG is a naturally occurring and beneficial soil fungus (*Paecilomyces lilacinus* strain 251) that controls a wide range of plant parasitic nematodes. MeloCon® WG has been shown in replicated field trials to control both southern root knot nematodes (*Meloidogyne incognita*) and stubby root nematodes (*Trichodorus* spp. and *Paratrichodorus* spp.), as well as many others. SoilGard® 12G is also a naturally occurring and beneficial soil fungus (*Gliocladium (Trichoderma) virens* strain GL-21) that controls a wide range of soil borne pathogens, including southern blight (*Sclerotium rolfsii*), *Fusarium* crown rot, and pepper blight (*Phytophthora capsici*). Replicated field trials using tomatoes with these products in conjunction with soil applied herbicides resulted in improved plant growth, increased survival, and increased yields, similar to methyl bromide and other chemical standards.

Yield loss associated with sheath blight disease of rice

C. A. HOLLIER (1), D. E. Groth (2)
(1) Department of Plant Pathology and Crop Physiology, LSU Agricultural Center, Baton Rouge, LA, USA; (2) Rice Research Station, LSU Agricultural Center, Crowley, LA, USA
Phytopathology 100:S201

Sheath blight is one of the most important rice diseases in the southern USA rice-producing area. Yield loss estimates are made annually but accurate measurements need to be taken. Fungicides were used a tool to influence sheath blight development in small plots. Applications were made with the aid of CO_2– pressurized sprayers delivering 93L/ha of solution at various times to halt or delay disease development to determine the affect of disease development on yield at different growth stages. Early disease development on enclosed canopy rice reduced yield greater than epidemics that were halted until late stages of crop development. Yield losses ranged from 7.83% for heading stage development (late developing disease), 16.65% for boot stage development (intermediate developing disease) to 28.63% for green ring stage development (early developing disease).

Management strategies for Pierce's disease: An increasing threat to grape production in the southern US

D. HOPKINS (1)
(1) University of Florida, Mid-Florida REC, Apopka, FL
Phytopathology 100:S201

Pierce's disease (PD) of grapevine, caused by *Xylella fastidiosa*, affects grape production across the southern U.S. and is especially damaging in the Southeast, where it is the primary factor limiting the development of a grape industry based on the high-quality *Vitis vinifera* grape. PD is increasing in severity in the southeastern U.S. as a result of warmer winter temperatures, increasing the risk of PD in the Piedmont region. Currently, the only feasible control for PD in most of the southeastern U.S. is genetic plant resistance. Management strategies currently being used or tested include vector control, removal of reservoir hosts, various transgenes in grape cultivars or rootstocks, and biological control with benign strains of *X. fastidiosa*. In Temecula CA, an area wide leafhopper vector management program has been credited with saving the grape industry from a 100% loss to PD. Several transgenic grape lines are ready for field trials to evaluate resistance to PD, including lines containing transgenes for anti-microbial proteins, for programmed cell death, and for diffusible signal factor. In Florida, injection of a benign strain (EB92-1) of *X. fastidiosa* into transplants has controlled PD in a *Vitis vinifera* cv. Cabernet Sauvignon planting for 13 years. This control could be available for commercial use in 2–3 years.

Occurrence of boscalid-insensitive isolates of *Didymella bryoniae* in commercial watermelon fields in South Carolina

A. P. KEINATH (1), E. Fillippeli (1)
(1) Clemson Coastal REC, Charleston, SC, USA
Phytopathology 100:S201

Insensitivity to boscalid in *Didymella bryoniae*, causal agent of gummy stem blight on cucurbits, was found in Georgia in 2007. In 2009, isolates were collected in South Carolina from watermelon leaves with symptoms of gummy stem blight in four commercial fields in three counties and one research plot. All five sites had been sprayed with a boscalid-pyraclostrobin premixture (Pristine) in 2009 and in prior years. Sensitivity of these isolates to boscalid was compared to sensitivities of isolates that had never been exposed to boscalid collected from watermelon in 1998 or from muskmelon in 2002 and isolates previously exposed to Pristine collected from watermelon in 2005. When possible, the 2009 isolates were collected from sites sampled in 2005 and 1998. Suspensions of conidia and ascospores of 57 isolates were placed on water agar amended with 0, 0.01, 0.10, 1.0, or 10.0 mg/l technical grade boscalid. On each plate, spore germination was counted after 24 h. Relative percentage germination (germination on amended media/germination on nonamended medium) was regressed against the logarithm of fungicide concentration to calculate EC_{50} values. Insensitive isolates were found at all five sites sampled in 2009. Of 30 isolates collected in 2009, 13 had EC_{50} values >10 mg/l, 11 had EC_{50} values >1 mg/l, and 6 had EC_{50} values <1 mg/l. EC_{50} values for all 27 isolates collected in 1998 to 2005 were <1 mg/l. Spores of 27 of the 30 isolates collected in 2009 germinated on agar amended with 10.0 mg boscalid per liter compared to only 1 of the 27 isolates collected in 1998 to 2005. Ten isolates collected in 2009 were insensitive to 10 mg/l, based on germination of >50% of conidia and ascospores on amended medium. Isolates of *D. bryoniae* from South Carolina are now insensitive to both pyraclostrobin and boscalid.

Baseline sensitivity to fluopicolide in *Phytophthora capsici* isolates from the eastern United States

A. P. KEINATH (1), E. Fillippeli (1), M. K. Hausbeck (2), C. Kousik (3)
(1) Clemson Coastal REC, Charleston, SC, USA; (2) Michigan State University, East Lansing, MI, USA; (3) USDA, ARS, Charleston, SC, USA
Phytopathology 100:S201

Fluopicolide was registered in 2007 to control diseases caused by Oomycete pathogens such as *Phytophthora capsici* on cucurbits and peppers. In this study, 69 isolates of *P. capsici* from Michigan (24 isolates), South Carolina (17), Georgia (14), Florida (11), and North Carolina (3) recovered from watermelon (22), pepper (11), bean (10), squash (9), cucumber (6), or unknown hosts (11) were tested to determine their sensitivities to fluopicolide. In three assays, isolates were grown on V8 agar amended with technical grade fluopicolide dissolved in DMSO. For the mycelial growth assay, concentrations were 0, 0.03, 0.10, 0.30 and 1.0 mg/l. For the sporangia production assay, concentrations were 0, 0.03, 0.10, and 0.30 mg/l with a few isolates also tested at 0.01 mg/l. For the zoospore germination assay, isolates were initially tested at 0.10, 1.0, and 10.0 mg/l; some isolates then were tested at 0.03 or 31.6 mg/l. Percentage colony diameter, zoospore germination, and sporangia production relative to the nonamended control was regressed against the logarithm of fungicide concentration to calculate EC_{50} values. All isolates of *P. capsici* tested were sensitive to fluopicolide in all three assays. EC_{50} values for each assay were non-normally distributed. The median concentration was 0.28 (range 0.11 to 1.56), 0.04 (<0.01 to 0.14), and 2.08 (0.14 to 13.74) mg/l in the mycelial growth, sporangia production, and zoospore germination assays, respectively. The ratio between the least and most sensitive isolates was 14 for mycelial growth and sporangia production. For zoospore germination, the ratio was 98 across all isolates but ranged from 3 to 44 for isolates within states. For mycelial growth and zoospore germination, isolates from Michigan had a higher mean EC_{50} value than isolates from other states ($P < 0.05$). Zoospore germination was much less sensitive and sporangia production was much more sensitive to fluopicolide than mycelial growth was.

Redbud yellow ringspot disease: Thirty years of research

A. G. LANEY (1), R. C. Gergerich (1), I. E. Tzanetakis (1)
(1) University of Arkansas
Phytopathology 100:S201

In the 1970s a disease was found infecting eastern redbud, Cercis canadensis. Symptoms include chlorotic ringspots, oak-leaf, and vein chlorosis in mature leaves and are usually expressed early in the season. Previous work revealed the presence of virus-like double membrane-bound bodies in diseased plants. Similar bodies have been found associated with several diseases including rose rosette, high plains disease, fig mosaic, European mountain ash ringspot, and thistle mosaic. Recently, the genomes of the viruses associated with Fig mosaic (FMV) and European mountain ash ringspot (EMARaV) were sequenced, and found to be negative sense ssRNA viruses related to tospoviruses. We have obtained sequence information of a virus found in yellow ringspot diseased plants, provisionally named Redbud yellow ringspot-associated virus (RYRaV). Detection protocols have been developed and used in a survey of symptomatic redbud trees. RYRaV was found closely associated with diseased trees as more than 90% of tested material was infected with the virus. Potential field alternative hosts were surveyed and a several herbaceous hosts were inoculated mechanically and by grafting. Transmission studies using eriophyid mites are under way.

Genetic diversity of the *Sclerotinia homoeocarpa* population in Florida

D. LIBERTI (1), G. T. Cooper (1), J. A. Rollins (1), P. F. Harmon (1)
(1) University of Florida, Plant Pathology Department, Gainesville, FL
Phytopathology 100:S201

Dollar spot disease of turfgrass, caused by the fungus *Sclerotinia homoeocarpa*, is the most important turfgrass disease occurring world-wide on all cool and warm season turfgrass species. The ribosomal DNA (rDNA) sequences were obtained from twenty-six isolates collected from Floridian warm season turfgrass species including bermudagrass (*Cynodon dactylon* (L.) Pers.), seashore paspalum (*Paspalum vaginatum* Sw.), St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) and zoysiagrass (*Zoysia japonica* Steud.) and from the Floridian cool season turfgrasses, rough

bluegrass (*Poa trivialis* L.) and creeping bentgrass (*Agrostis palustris* Huds). Isolates were collected from 26 distinct golf courses and other turfgrass swards in 12 Florida counties between 2004 and 2009. These data plus 14 other *S. homoeocarpa* rDNA sequences from GenBank were subjected to phylogenetic analysis using the neighborg-joining method and choosing *Sclerotinia sclerotiorum* (Lib.) De Bary and *Poculum henningsianum* (Plottn.) T. Schumach. as outgroups. Phylogenetic reconstructions based on sequences of internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2) indicated that twenty out of twenty-six Floridian isolates clustered in a group that represents a newly identified biotype of *S. homoeocarpa*. Further characterization of this Floridian biotype is in progress.

Effect of southern root-knot nematode (*Meloidogyne incognita*) on cotton growth, yield and fiber quality

P. LU (2), R. C. Kemerait (2), C. D. Perry (2), R. F. Davis (1)
(1) USDA-ARS, Tifton, GA, USA; (2) University of Georgia, Tifton, GA, USA
Phytopathology 100:S202

Southern root-knot nematode (SRKN) (*Meloidogyne incognita*) is a major pest of cotton worldwide. Much research has been devoted to impact of SRKN on yield; less information is available regarding impact of SRKN on fiber quality. Objectives were to assess impact of SRKN on growth, yield, and fiber quality at five sites planted to cotton in Georgia in 2008 and 2009. Three sites were planted to DPL 555B/RR and two were planted to Fiber Max 9063B2F and Stoneville 4554B2RF. A randomized complete block design with 4-6 replications was used at each site. Risk management zones for SRKN were established at three locations based upon characteristics to include elevation, slope, soil electroconductivty and NDVI from bare soil reflectance. Nematicides (aldicarb, 3-6 lb/A), 1,3-dichloropropene (3-6 gal/A) and two seed treatment nematicides were used to create differential populations of SRN. Growth of the crop, soil populations of root-knot nematodes, and damage to the plants were assessed. Cotton growth and yields were often significantly and negatively correlated to root gall ratings, populations of nematode juveniles and the number of SRKN eggs extracted from root samples. However, most fiber quality parameters were not correlated to soil nematode populations or damage to the cotton plants. However, in fields with higher populations of SRKN, fiber quality properties tended to be more strongly correlated to nematode populations and subsequent root damage than in fields with lower populations. Often, plant growth, yield and fiber quality were significantly different between the high and low risk management zones; however the impact of SRKN populations was not always clear.

Quantitative modeling of the effects of temperature and wetness duration on germination and infection of cantaloupe by *Pseudoperonospora cubensis*

K. N. NEUFELD (1), P. S. Ojiambo (1)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC
Phytopathology 100:S202

Cucurbit downy mildew caused by *Pseudoperonospora cubensis* is considered the most damaging disease of cucurbitaceous crops worldwide. Three response surface models were developed based on independent experiments in which cantaloupe plants were inoculated with *P. cubensis* and exposed to a range of leaf wetness durations (2–24 h) and fixed temperatures (5–30°C) in growth chambers. Germination was assessed at the end of each wetness period and infection was recorded 5 days after inoculation as percent leaf area with chlorotic and necrotic symptoms. Models were evaluated for their ability to predict germination and infection of *P. cubensis*. Optimum germination and infection was observed at 16.5 and 20.6°C, respectively, while little germination or infection occurred at 5 or 30°C. Optimum infection for wetness periods 4–8 h was observed at t = 20°C, but wetness periods > 8 h had broader optimum curves. Model 1 of the form $f(w,t) = f(t)\bullet(1\text{-exp}\{-[Bw]^D\})$ resulted in smaller asymptotic standard errors and yielded higher correlations between observed and predicted germination and infection data than either model 2 of the form: $f(w,t) = A\{1\text{-exp}[-f(t)\bullet(w\text{-}C]^D\}$ or model 3: $f(w,t) = [1\text{-exp}(-Bw)^2] / \cosh[(t\text{-}F)G/2]$. Models 1 and 2 had non-significant lack-of-fit statistics while a lack-of-fit test was significant for model 3 for both germination and infection data. These models accounted for up to 98% of the total variation in the data. Risk threshold charts were developed to estimate the potential risk of cucurbit downy mildew epidemics in the field based on temperature and duration of leaf wetness.

Efficacy of strobilurin fungicides and host resistance for control of gray leaf spot of corn

M. A. NEWMAN (1)
(1) University of Tennessee, Jackson, TN, USA
Phytopathology 100:S202

Gray leaf spot (GLS) of corn, caused by *Cercospora zeae-maydis* is a common foliar disease that reduces corn yields in Tennessee and many other states. Two strobilurin fungicides (azoxystrobin and pyraclostrobin) have shown a high degree of control of GLS in tests conducted over the last three years (2006–2008) at the Research and Education Center at Milan, TN. Each fungicide was sprayed at (01.1 kg/ha a.i.) with Penetrator Plus @ 0.125% v/v as an adjuvant. Four-row plots 30' long were randomized and replicated four times. Rows were on 30" centers and planted no-till in a field infested with GLS. The following three Pioneer corn hybrids with different levels of resistance to GLS were used: susceptible P 32T22, moderately susceptible P 33R76 and tolerant P 33V14. Each fungicide was sprayed once over the top at the VT growth stage (tassel) in 20 gallons of water per acre. Yield increases over the untreated control for the three-year period were significantly greatest for the susceptible hybrid for both fungicides. For the susceptible hybrid, the average three-year yield increase over the untreated was 1613 kg/ha with azoxystrobin and 1545 kg/ha with pyraclostrobin. The average three-year yield increase using the moderately susceptible hybrid was 1210 kg/ha with azoxystrobin and 470 kg/ha with pyraclostrobin. For the tolerant hybrid, the average three-year increase in yield was 538 kg/ha with azoxystrobin and 403 kg/ha with pyraclostrobin respectively. These results indicate that spraying strobilurin fungicides can increase corn yields, especially on the more GLS susceptible hybrids.

Screening Gulf Coast forest species for susceptibility to *Phytophthora ramorum*

J. A. PREUETT (2), D. J. Collins (2), T. L. Widmer (1), D. G. Luster (1)
(1) USDA/ARS Foreign Disease-Weed Science Research Unit, Fort Detrick, MD, USA; (2) Urban Forestry Program, Southern University and A&M College, Baton Rouge, LA, USA
Phytopathology 100:S202

Phytophthora ramorum, the causal agent of sudden oak death, is an emerging pathogen of California oak woodlands. This pathogen poses a threat to woody plants in many areas of North America, due to the broad host range of the pathogen and the wide distribution of hosts. The US Gulf Coast area is considered a high risk due to the suitable climate, but the question remains whether Gulf Coast woody understory species represent possible hosts for the pathogen. The following woody plant species, native to the Gulf Coast forest: yaupon (*Ilex vomitoria*), spice bush (*Lindera benzoin*), southern magnolia (*Magnolia grandiflora*), and eastern baccharis (*Baccharis halmifolia*) were tested for their reaction to *P. ramorum*. This study was conducted at the USDA/ARS plant pathogen containment greenhouse facility at Ft. Detrick, MD. Foliage of four test plants was inoculated with 50,000 zoospores per ml until the foliage was completely wet. The test was repeated three times for each plant species. Inoculated plants were placed in a dew chamber at 20°C for 4 days. After this incubation period, the leaves were detached, scanned on a flatbed scanner, and the leaf lesion areas were assessed for disease using ASSESS 2.0 software. Yaupon and southern magnolia appeared to be susceptible to *P. ramorum*. The average percentage of lesion leaf area was 4.9, 0.2, 28.1 and 32.1% for inoculated spice bush, eastern baccharis, yaupon and southern magnolia plants, respectively. This is compared to 1.2, 0.4, 0.1 and 0.6%, respectively, for the non-inoculated controls. We plan to continue this research to analyze additional Gulf Coast forest plant species for reaction to *P. ramorum*.

Characterization of rice blast resistance gene *Pi-z(t)* in rice germplasm using DNA markers and pathogenicity assays

M. ROY-CHOWDHURY (1), Y. Jia (2), A. Jackson (2), M. Jia (2), R. Fjellstrom (2), R. Cartwright (1)
(1) University of Arkansas, Cell and Molecular Biology Program, Fayetteville, AR, USA; (2) USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, AR, USA
Phytopathology 100:S202

The *Pi-z(t)* gene in rice confers resistance to a wide range of races of the rice blast fungus, *Magnaporthe oryzae*. The objective of the present study was to identify *Pi-z(t)* in 131 worldwide rice germplasm using DNA markers and pathogenicity assays. Four simple sequence repeat (SSR) markers (RM527, AP4791, AP5659-1, AP5659-5) closely linked to *Pi-z(t)* were first used to predict the existence of *Pi-z(t)* in rice germplasm and results were verified using pathogenicity assays with an avirulent IE1k / two virulent races, IB33 and IB49. A total of 98 germplasm containing one to four SSR alleles for the *Pi-z(t)* gene was found to be resistant to IE1k and susceptible to IB33 and IB49, suggesting these germplasm contain different *Pi-z(t)* haplotypes. Eighteen germplasm containing one to four SSR alleles were found to be resistant to all three races, suggesting the presence of other *R* gene(s) in addition to *Pi-z(t)*. Five germplasm containing three to four SSR alleles were found to be susceptible to all races, indicating the absence of the *R* gene(s) or presence of non functional components of *Pi-z(t)* in these germplasm. Six germplasm containing one to four SSR alleles, with one having novel alleles,

were found to be resistant to IB49 and IE1k but susceptible to IB33, suggesting that other *R* gene in these germplasm confer resistance to IB49. Three germplasm containing two to three SSR alleles were found to be resistant to IB33 and IE1k and susceptible to IB49, suggesting the presence of additional *R* gene(s) to IB33 in these germplasm. Finally, one germplasm with novel SSR alleles was found to be resistant to all races, suggesting the presence of *Pi-z(t)* independent *R* gene(s) in this germplasm. These characterized germplasm should be useful for genetic studies and marker assisted breeding for improving blast resistance worldwide.

Zebra chip of potato: Current status and future outlook

C. M. RUSH (1), D. C. Henne (1), F. Workneh (1) and N. Gudmestad (2)
(1) Texas AgriLife Research, Amarillo, TX; (2) North Dakota State University, Fargo, ND
Phytopathology 100:S203

In Texas, potatoes are grown in the Rio Grande Valley, the Winter Garden area near San Antonio, and the Panhandle. In 2000, potatoes from the lower Rio Grande Valley displayed brown necrotic flecks and streaking of the medullary rays. In fry tests, chips from these tubers exhibited dark brown blotches and stripes, which were initially referred to as Texas Defect, but later renamed Zebra Chip (ZC). Zebra Chip affects all market classes of potatoes by reducing yield and quality, and now has been identified in California, Colorado, Kansas, Nebraska, New Mexico and Wyoming. In areas where ZC has become established, it is the most economically important impediment to profitable potato production. Recently, a phloem-restricted proteobacterium, *Candidatus* Liberibacter, has been associated with ZC. Two newly named species, *Ca.* Liberibacter psyllaurous and *Ca.* Liberibacter solanacearum, have been reported as etiological agents of the disease. Both are transmitted by the potato psyllid *Bactericera cockerelli,* but sequence analysis suggests that the two are likely the same species. However, slight differences between the two suggest the possibility of strains. No genetic resistance to ZC has been identified, insecticides for psyllid control are often ineffective, and factors which impact disease epidemiology are largely unknown. In response to this national threat to the potato industry, a multidisciplinary, multistate team of researchers and extension specialists initiated a program with the goal of reducing losses from ZC to economically sustainable levels by development of a comprehensive, environmentally responsible disease management program. To accomplish this, an advisory board of farmers and representatives from ag-industry, together with the participating scientists, identified seven priority focus areas (Disease Etiology and Vector/Pathogen Diversity, Epidemiology, Pest Management, Breeding, Economics, Risk Assessment and Technology Transfer), each with a number of specific objectives, which together constitute an integrated systems approach to resolving the ZC problem.

Etiology of zoysiagrass diseases in northwest Arkansas

T. N. SPURLOCK (1), E. A. Milus (1)
(1) University of Arkansas, Fayetteville, AR, USA
Phytopathology 100:S203

The increased use of zoysiagrass in Northwest Arkansas has raised awareness of its susceptibility to a range of pathogens. The most destructive and widespread disease is large patch caused by *Rhizoctonia solani* AG 2-2 (LP). The disease occurs on warm season turfgrasses in the transition zone and is very destructive to zoysiagrass in Northwest Arkansas. Symptoms include irregular patches of dying turf up to several meters in diameter with distinct lesions on the leaf sheaths and stems. Management of large patch with fungicides is expensive and control is variable, possibly indicating that other microorganisms are associated with the disease. The objectives of this study were to determine if other microorganisms isolated from large patch areas contribute to disease severity. Fungi and oomycetes were isolated from leaf sheaths, stems, and rhizomes of zoysiagrass with large patch symptoms from fairways of three golf courses in Northwest Arkansas. Isolates were grouped by morphological characteristics and frequency of isolation recorded. *R. solani* AG 2-2 (LP) was the most frequently isolated fungus from all sampling locations. Isolates representative of other morphological groups were tested for pathogenicity on *Zoysia japonica* cv. Meyer and *Zoysia matrella* cv. Cavalier. Individually, *R. solani* and *Gaeumannomyces graminis* var. *graminis* increased the proportion of shoots with lesions (DS) and decreased biomass (B) over the non-inoculated control in both cultivars. When zoysiagrass was inoculated with another isolate in addition to *R. solani*, a *Fusarium* isolate and a sterile white basidiomycete caused significantly less disease (DS and B) in Cavalier than *R. solani* alone. However, the addition of a *Pythium* isolate with *R. solani* decreased B over *R. solani* alone in both cultivars and DS in Meyer. Disease severity and growth for the combination of *R. solani* and *G. graminis* var. *graminis* was not significantly different from these pathogens inoculated singularly.

Comparison of seed treatments for control of soybean seedling diseases in field soil at three temperatures

K. E. URREA (1), J. C. Rupe (1), C. S. Rothrock (1)
(1) University of Arkansas
Phytopathology 100:S203

Seedling diseases frequently reduce seed germination, seedling emergence, stand, vigor, and yield, and sometimes require replanting. Since seedling disease severity depends on the pathogens present in the soil and the environmental conditions, evaluation of seed treatments and cultivars in the field can be very erratic. The objectives of this research were to evaluate selective and broad spectrum fungicide seed treatments and cultivars in field soil under controlled environmental conditions. Three cultivars were treated with six fungicide treatments or not treated and planted in soil collected from two fields in April, May and June in 2008 and 2009. Tests were conducted in growth chambers at 21°C (April soil), 25°C (May soil) or 28°C (June soil) and soils were watered when matric potentials reached –30 J/kg. After two weeks, the tests were rated for stand, root rot and plant growth and isolations were made from the roots. In 2008, seed treatments resulted in greater stands than the control at all three temperatures only for the cultivar Archer. In 2009, seed treatments resulted in a significant increase in stands for low and high quality seed of the cultivar Hutcheson, but not for HBK4924. Allegiance® was the most effective fungicide tested across all the temperatures in increasing plant stands. For all temperatures and soils, *Pythium sylvaticum* followed by *Fusarium oxysporum* were the most frequently isolated pathogens from roots. These results indicate that by controlling environmental conditions seed treatment fungicides and the importance of different seedling pathogens can be efficiently evaluated.

Cellular mechanisms that indicate needle health of seedlings of loblolly pines

C. WALKINSHAW (1)
(1) USDA Forest Service, Pineville, LA
Phytopathology 100:S203

Health of needles on seedlings is readily apparent in pines. Symptoms of disease are easy to recognize by observing the whole leaf or thin sections of needle tissue. In this study histology techniques were used on both healthy and diseased needles that were fixed, sectioned and examined for tannin and other variables, including necrosis of resin ducts. The largest variation in number of starch grains and cells with excess tannin occurred in the resin ducts. These were lined with epithelial cells and had an outer layer of parenchyma cells that were often torn during normal growth. Sporadic healing occurred in areas near phenol cells. Energy for repair of these cells appeared to originate from the collenchyma. Bands of phloem within the needle traces had high starch content. Phenol oxidase, acid phosphatase and peroxidase enzymes as measured by histochemical techniques, combined to hydrolyze cell contents. These observations describe the biology of a number of cellular changes that are associated with susceptibility of loblolly pine needles to decline in the greenhouse and field.

Development of a screening protocol for assessing baseline sensitivity to fungicides for *Phakopsora pachyrhizi*, the soybean rust pathogen

N. A. Ward (1), R. W. SCHNEIDER (1), C. G. Giles (1), C. L. Robertson (1)
(1) Department of Plant Pathology & Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA
Phytopathology 100:S203

Soybean rust, caused by *Phakopsora pachyrhizi*, was first discovered in the continental United States in November 2004. Since then it has been detected throughout the U.S. and Ontario, Canada. The disease has been particularly severe in the Southeast where producers are forced to apply fungicides. These continual applications have the potential to select for fungicide resistant strains of the rust pathogen, and these strains could easily overwinter along the Gulf Coast on kudzu and other alternative hosts. The purpose of this study was to develop a sensitive and repeatable assay for establishing baseline sensitivity concentrations for two classes of fungicide chemistry, namely triazoles and strobilurins. The following fungicides were included in this study: tetraconazole (Domark®), flutriafol (Topguard®), azoxystrobin (Quadris®), and pyraclostrobin (Headline®). Freshly produced urediniospores were collected by brushing and discarding existing spores from infected leaves with an artist's paintbrush. These leaves were then incubated in a moist chamber (25°C) for 2 days after which hyaline urediniospores were produced in abundance on the lower leaf surface. These leaves were placed (lower surface down) on a grid suspended over a plastic petri dish, and the upper leaf surface was gently tapped. This dislodged the urediniospores into the dish, and the spores were then dabbed with the brush to break apart clumps. Water agar plates amended with various concentrations of the fungicides were inoculated by touching spores in the spore collection plates with the tip of the brush and then touching the surface of the amended agar plates with

the brush. Spore germination was assessed after incubation for 4 hours in the dark at 25°C.

Pod yield of peanut breeding lines from fields infested with *Sclerotinia minor* or *Verticillium dahliae*

J. E. WOODWARD (1), M. R. Baring (3), C. E. Simpson (4), T. A. Baughman (2)
(1) Texas AgriLife Extension Service, Lubbock, TX, USA; (2) Texas AgriLife Extension Service, Vernon, TX, USA; (3) Texas AgriLife Research, College Station, TX, USA; (4) Texas AgriLife Research, Stephenville, TX, USA
Phytopathology 100:S204

Diseases such as Sclerotinia blight (*Sclerotinia minor* Jagger) and Verticillium wilt (*Verticillium dahliae* Kleb.) can drastically reduce peanut (*Arachis hypogaea* L.) yields in Texas. Field trials were conducted to evaluate the performance of advanced peanut breeding lines in a field naturally infested with *S. minor*. Pod yields were increased by 2457, 1391, 1226 and 981 kg ha^{-1} for breeding lines TX-3, TX-2, TX-1 and TX-4, respectively, when compared to the commercial standard 'Flavor Runner 458'. Yield for these breeding lines were equivalent to or greater than that of the partially resistant cultivar 'Tamrun OL07'. Separate trials were conducted on the Southern High Plains to evaluate the performance of breeding lines TX-3, TX-4, TX-5 and TX-6 in fields infested with *V. dahliae*. Pod yields were greatest for breeding line TX-3 and 'Flavor Runner 458', 5038 and 4960 kg ha^{-1}, respectively, whereas yield was lowest for breeding line TX-5 (3966 kg ha^{-1}). Pod yields for the cultivars 'McCloud', TX-6, 'Tamrun OL02' and 'Tamrun OL07' where intermediate ranging from 4476 to 4254 kg ha^{-1}. Results from these studies indicate that there are varying levels of resistance to *S. minor* and *V. dahliae* among the breeding lines evaluated. Breeding line TX-3 may be suitable for fields co-infested with both pathogens.

Characterization of interacting genes with the rice blast fungus avirulence gene *AVR-Pita*

J. Xing (1), S. Lee (2), Y. JIA (2), L. Yuan (1)
(1) Central South University, Changsha, P.R. China; (2) Dale Bumpers National Rice Research Center, Stuttgart, AR, USA
Phytopathology 100:S204

The *AVR-Pita* gene in *Magnaporthe oryzae* determines the efficacy of the *Pi-ta* blast resistance gene. *AVR-Pita* encodes a predicted metalloprotease with 223 amino acids. *AVR-Pita*$_{176}$ with deletion of 57 amino acids at the amino terminus was previously shown to be involved in *Pi-ta* mediated blast resistance as a putative effector protein. In order to study the role of *AVR-Pita* in fungal pathogenicity and blast resistance, *AVR-Pita*$_{176}$ was used as bait to identify interacting genes from a yeast two-hybrid library constructed using mRNAs isolated from a U.S. tropical japonica cultivar Katy leaves at different time points after inoculation with *M. oryzae*. Identified AVR-Pita interacting proteins will be verified using *in Vitro* binding techniques. In addition, three predicted proteins, AVR-Pita$_{223}$, AVR-Pita$_{176}$, and AVR-Pita$_{166}$ will be used to examine interaction specificity in the yeast two-hybrid assays. The roles of AVR-Pita interacting proteins in fungal pathogenicity and blast resistance will be investigated and progress will be presented.

Histopathology of 'rapid blight', a disease caused *Labyrinthula terrestris* on cool-season turfgrasses

K. YADAGIRI (1), J. L. Kerrigan (1)
(1) Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC, USA
Phytopathology 100:S204

Rapid blight is a disease on cool-season turfgrasses, caused by a microorganism known as *Labyrinthula terrestris*. Symptoms of rapid blight include water-soaked lesions and browning or bronzing of foliage that lead to yellowing and death of the infected turf. So far, eleven states in the US on both coasts have reported rapid blight, in addition to other countries including the United Kingdom, Spain and Argentina. Saline irrigation water and soil are favorable for disease causation. Rapid blight is more severe on salt-sensitive varieties of turfgrasses that are mostly cool-season turfs, such as rough bluegrass (*Poa trivialis*), perennial ryegrass (*Lolium perenne*), annual bluegrass (*Poa annua*) and colonial bentgrass (*Agrostis tenuis*). *Labyrinthula terrestris* is an unusual pathogen on turf; it belongs to a group of organisms commonly referred to as marine net-slime molds, which have been primarily known to cause diseases on sea grasses. Details of the host-pathogen interactions of *Labyrinthula terrestris* on turfgrasses have not been investigated. We are, therefore, documenting the infection processes and life cycle using light and electron microscopy to better understand the pathogenicity of this organism and, ultimately, apply these finding to provide better means of controlling rapid blight disease.

Epidemiology of soybean rust in soybean sentinel plots in Florida

H. M. YOUNG (2), J. J. Marois (2), D. L. Wright (2), D. F. Narváez (1), G. K. O'Brien (2)
(1) Monsanto Co., St. Louis, MI, USA; (2) University of Florida, NFREC, Quincy, FL, USA
Phytopathology 100:S204

Since its discovery in 2004 in the Southeastern United States, soybean rust (SBR) severity has been variable from year to year. It is important to understand the epidemiology of the pathogen in Florida as it may serve as an inoculum source for other areas of the country. This study examined the incidence and severity of SBR in relation to prevailing weather data, growth stage, and maturity group (MGIII, MGV, MGVII) in soybean plots (15 m square) across north Florida that were part of the national sentinel plot network from 2005 through 2008. On average, plots became infected 30 days earlier in 2008 than 2005. Precipitation was the principle factor affecting disease progress, where disease increased rapidly after rain events and was suppressed during dry periods. In 2008, there was a significant increase in disease incidence and severity as reflected in the area under the disease progress curve. This was associated with the occurrence of Tropical Storm Fay, which deposited up to 290 mm of water in the plot locations. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this pathosystem.

Effect of temperature on latent period of Stagonospora nodorum blotch on winter wheat under field conditions

A. D. ZEARFOSS (1), C. Cowger (2), P. S. Ojiambo (1)
(1) Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA; (2) USDA-ARS, North Carolina State University, Raleigh, NC, USA
Phytopathology 100:S204

Stagonospora nodorum is the causal agent of Stagonospora nodorum blotch (SNB) and yield losses from severe disease epidemics can be as high as 50%. To establish a model for SNB development based on the effects of temperature on pathogen latent period and life cycle relative to the host, batches of two winter wheat cultivars (AGS 2000 and USG 3209) were inoculated with pycnidiospores of *S. nodorum* at weekly intervals over a 1 year period. After an incubation period of 72 h, plants were exposed to field conditions where prevailing temperatures ranged from 5°C to 28°C with a mean batch temperature of 9°C to 24°C. Latent period until the first visible symptoms ranged from 8 to 34 days. The relationship between development of lesions with pycnidia and accumulated thermal time will be described using a shifted cumulative gamma distribution model using estimated base temperatures. These results will provide valuable data that link crop growth models with the progress of SNB and facilitate the establishment of disease development models for use in timing fungicide applications.

2010 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division meeting in Ocean City, Maryland, March 24–26, 2010. The abstracts are arranged alphabetically, by first author's name.

Host range determination of *Colletotrichum gloeosporioides* f. sp. *salsolae*, a biological control agent of tumbleweed: From BLUPs to biomass loss
D. K. Berner (1), C. A. CAVIN (1)
(1) USDA, ARS, FDWSRU
Phytopathology 100:S205

Host range tests were conducted with *Colletotrichum gloeosporioides* f. sp. *salsolae* (CGS) in quarantine to determine whether the fungus is safe to release in N. America for biological control of tumbleweed (*Salsola tragus* L., Chenopodiaceae). Ninety two accessions were analyzed from 19 families and 10 tribes within the family Chenopodiaceae. These included 62 genera and 120 species. Disease reaction data were combined with a relationship matrix derived from internal transcribed spacer DNA sequences and analyzed with mixed model equations to produce Best Linear Unbiased Predictors (BLUPs) for each species. Twenty nine species from 7 closely-related Chenopodiaceae tribes had significant levels of disease severity as indicated by BLUPs. Most species in the genus Salsola, which are all introduced and weedy, were very susceptible and damaged by CGS. Of the 29 susceptible species, 10 native or commercially important species in N. America were identified as needing additional tests to determine the extent of any damage caused by disease. These additional tests were done by inoculating the non-target species of concern with CGS and weighing oven-dried shoots and roots of non-inoculated and inoculated plants one month after inoculation. The shoots and roots of each plant were scanned and the surface areas determined with image analysis software. The damage to the shoots and roots of each plant were standardized by dividing surface area by the corresponding dry weights to arrive at area per unit weight. Average differences in standardized plant damage between inoculated and controls for each plant species were combined with corresponding disease ratings and analyzed by principal component analysis. Results showed that most of the non-target species clustered as not-damaged while the target and several related weedy species were heavily damaged. Three non-target species were moderately damaged, but these species were either perennial or not ecologically sympatric with tumbleweed.

Extract of the brown seaweed *Asophyllum nodosum* and silicon reduce plant death due to *Fusarium* spp. of cucurbits
G. E. Brust (3), R. E. ROSS (1), J. Jayaraj (2)
(1) Acadian Seaplants LLC, Dartmouth, NS, Canada; (2) Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad; (3) University of Maryland, Upper Marlboro, MD, USA
Phytopathology 100:S205

Crop losses due to *Fusarium* spp. are important to cucurbit growers along with an increasing interest in natural ways to improve disease resistance. Extracts of the brown seaweed, *Ascophyllum nodosum*, and products containing silicon have both been shown to promote disease resistance in many crops. In a 2008 watermelon trial located in Upper Marlboro, MD, *Fusarium solani* symptoms were suppressed by extracts of *A. nodosum*. At the final rating, 30% of the watermelon plants were dead from this pathogen in the control plots vs. 10% in *A. nodosum* extract treatments. A second study was implemented in 2009 on Gladiator Pumpkins. Calcium silicate and *A. nodosum* extract were applied to pumpkins grown in a field known to have *Fusarium* spp. infected squash three years prior. At the final rating, 24.6% of the pumpkin plants were dead in the control plots vs. 19.2% in the silicon plots, 13.6% in the *A. nodosum* extract treatment, and just 6.1% in the plots with both calcium silicate and *A. nodosum* extract. These field trials were further supported by two greenhouse studies where applications *A. nodosum* extract applied to cucumber plants reduced incidence of *Fusarium oxysporum* and enhanced the activities of plant defense-related enzymes including chitinase, beta-1,3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and lipoxigenase as well as elevated levels of total phenols compared to the control. The jasmonic acid pathway has been found to be very important in plant defense responses elicited by *A. nodosum*. Pathogens that are inhibited by the jasmonic acid pathway are often necrotrophs such as *Fusarium* spp. *A. nodosum* extract may offer a valuable tool to improve the health and productivity of cucurbits.

Detection and distribution of *Bean pod mottle virus* in soybean and beetle vectors in eastern Virginia
M. E. CASSELL (1), T. P. Kuhar (2), P. B. Schultz (3), S. A. Tolin (1)
(1) Virginia Tech, Blacksburg, VA; (2) Virginia Tech-ESAREC, Painter, VA; (3) Virginia Tech-HRAREC, Virginia Beach, VA
Phytopathology 100:S205

Bean pod mottle virus (BPMV) (genus *Comovirus*; family *Comoviridae*) is a reemerging disease of soybeans. Vectored by the Bean leaf beetle (*Cerotoma trifurcata*), this virus has become prevalent on the Eastern Shore of Virginia. In tissue blot immunoassays (TBIA) of soybean sentinel plots for the Legume IPM-PIPE in 2007, BPMV was detected at a high incidence at the Eastern Shore station, but not at the Tidewater station. In 2008, beetles collected at the Eastern Shore station were 80% positive for BPMV by TBIA, but all non-soybean legumes tested were TBIA-negative. In a systemic survey in 2009, BPMV was detected by TBIA in 16 of 42 soybean fields from the southern tip of the Eastern Shore to southern Maryland. Up to 100% of the beetles collected from 24 of 38 Virginia fields were positive for BPMV by ELISA of individual beetles. Infectious virus was recovered from beetle extracts prepared for ELISA. In 2009, an outbreak of BPMV was also detected in two counties in the Northern Neck of Virginia. The primary inoculum of BPMV remains unknown. Sampling is being conducted on the Eastern Shore to locate plants that might serve as an early season source of BPMV for acquisition by overwintering or first generation beetles.

Impact of mowing and fertility practices on weed species and brown patch dynamics in rhizomatous tall fescue
M. A. CUTULLE (3), D. McCall (2), B. Horvath (1), J. Derr (3)
(1) University of Tennessee, Knoxville, TN; (2) Virginia Tech, Blacksburg, VA; (3) Virginia Tech, Virginia Beach, VA
Phytopathology 100:S205

Tall Fescue (*Festuca arundinacea*) is a commonly utilized turfgrass in the temperate and transition zone areas of the United States. It establishes quickly, requires moderate amounts of nitrogen, and is resistant to most diseases. However, during hot humid summers, tall fescue is under stress and is susceptible to *Rhizoctonia solani* infection. The resulting disease, referred to as brown patch, causes turf thinning, leading to encroachment from weeds

such as bermudagrass (*Cynadon dactylon*). Cultural practices such as fertility and mowing height may impact bermudagrass encroachment and brown patch disease in tall fescue. Improved brown patch control may result in lower weed infestations. Two mowing heights (5 and 10 cm), three levels of fertility (49, 171, and 220 kg of nitrogen annually per hectare), and preemerge herbicide application (ronstar or no herbicide applied in 2009 only) were evaluated in an established stand of 'RTF' tall fescue. Three plugs of common bermudagrass were planted in each plot in May 2008. Data collected monthly included weed composition and density, bermudagrass diameter, brown patch severity, and turf quality. The experiment was repeated in May of 2009. Mowing height had a significant effect on bermudagrass in year one and year two. A higher mowing height resulted in less bermudagrass encroachment. Fertility did not have an effect on bermudagrass diameter. In July and August, southern crabgrass (*Digitaria ciliaris*) density was much greater in the 5 cm mowing height plots. Tall fescue cover was significantly reduced in the 5 cm mowing treatment due to weed competition but was acceptable at the 10 cm height. Higher fertility resulted in increased brown patch severity. However, these plots recovered quickly when weather was cooler and dryer. The same trends were observed in year two, though incidence of brown patch was greater in year two due to the increased precipitation.

A new phytoplasma lineage is associated with diseased juniper (*Juniperus occidentalis*)

R. E. DAVIS (1), E. L. Dally (1), Y. Zhao (1), I.-M. Lee (1), R. Jomantiene (2), A. J. Detweiler (3), M. L. Putnam (4)
(1) USDA-Agricultural Research Service, Beltsville, MD, USA; (2) Nature Research Center, Vilnius LT-08406, Lithuania; (3) Oregon State University Deschutes County Extension, Redmond, OR, USA; (4) Oregon State University Plant Clinic, Corvallis, OR, USA
Phytopathology 100:S206

Phytoplasmas are wall-less, prokaryotic plant pathogens that are spread from plant-to-plant by insects and are the cause of diseases in a wide range of plant species that include angiosperms and gymnosperms. Worldwide, work is underway to determine the possible association of phytoplasmas with plant diseases of unsolved cause, in order to devise disease control and quarantine measures based on knowledge of the pathogens involved. The present work focused on a disease (juniper witches' broom, JunWB) of *Juniperus occidentalis*, a native tree indigenous to parts of western USA. Amplification of ribosomal RNA gene sequences (rDNA) in polymerase chain reactions (PCRs) primed by phytoplasma-universal primers indicated that a phytoplasma was associated with the disease. Nucleotide sequences of the rDNA were analyzed using a computer-based interface, *i*PhyClassifier, to obtain virtual RFLP patterns of 16S rDNA; the results indicated that JunWB phytoplasma represented a new lineage in the pigeon pea witches' broom phytoplasma group (16SrIX). The findings expand the known biodiversity of phytoplasmas infecting conifers and raise the question of whether *J. occidentalis*, previously undescribed as a phytoplasma host, could play a role in the spread of phytoplasmal disease potentially damaging to forest and/or landscape conifers in North America.

Effects of exogenous indole-3-acetic acid on transcriptional reprogramming of hormone signaling and metabolism genes in potato purple top phytoplasma-infected tomato plants

Y. DING (1), W. Wei (1), W. Wu (1), Y. Jiang (1), R. E. Davis (1), Y. Zhao (1)
(1) Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD
Phytopathology 100:S206

Phytoplasmas are plant pathogenic bacteria that lack a cell wall. Plants infected by phytoplasmas exhibit various symptoms indicative of disrupted hormonal balance. Observations that exogenous application of auxins on aster yellows phytoplasma-infected periwinkle plants could induce symptom remission or even phytoplasma elimination further point to crucial roles of plant hormones in phytoplasma pathogenesis. The present study was designed to gain an insight into expression profiles of plant hormone signaling and metabolism genes in healthy vs phytoplasma-infected plants, and to examine whether exogenously applied indole-3-acetic acid (IAA, a naturally occurring auxin) would modify the expression patterns of these genes. 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. Our preliminary data revealed that, following graft inoculation of plants with PPT phytoplasma, expression patterns of a putative IAA biosynthesis gene and an F-box protein-encoding gene responsible for IAA signaling were altered. Exogenously applied IAA was able to reverse the course, bringing expression of the two genes to the levels comparable to those in healthy and mock-inoculated tomato plants. The findings provide a clue to understanding mechanisms of phytoplasma pathogenesis and exogenous auxin-induced phytoplasmal disease remission.

The science and art of photography for art and science

J. D. EISENBACK (1)
(1) Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA
Phytopathology 100:S206

Several art historians have noted that paintings suddenly improved in detail and realistic representation sometime around 1420 AD. This remarkable enhancement of drawings and paintings was credited to use of a convex mirror to project a scene onto a canvas. As the technology of glass production improved, conventional lenses were made for projecting images onto a canvas inside a large dark room, called a camera obscura. In the early 1800s light sensitive paper replaced the canvas and pigments, and the photograph was born. The camera obscura was downsized to the more mobile camera, a small box with a lens and a holder for light sensitive film. Cameras and photography became incorporated into nearly every facet of human activity, including scientific documentation. The discovery of the photovoltaic effect by Albert Einstein initiated the development of digital cameras in the late 1900s. Digital photography allows images to be readily manipulated in several ways, including high dynamic range (HDR) photography, multiple focus photography, and the production of megapixel mosaic photographs that can be utilized for art and science.

Effect of aging on survival and heat tolerance of anhydrobiotic seed-gall nematode, *Anguina agrostis*

J. D. EISENBACK (1), C. W. Roane (1), W. Ma (1)
(1) Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA
Phytopathology 100:S206

A seed gall nematode, *Anguina agrostis*, was found parasitizing redtop creeping bentgrass (*Agrostis stolonifera*), that over winters in an anhydrobiotic state. Infested grass seed was collected on Aug. 24, 2003 and Aug. 16, 2009 from a naturally infested site on Butt Mountain Lookout, near the fire watchtower, in Giles County, Virginia. Samples that were stored in an open plastic bag in a laboratory cabinet for more than 5 years were compared to freshly collected specimens. Seed galls were soaked in tap water for 24 hr. to evaluate the survival of juvenile nematodes. Additional seed galls were exposed to high temperatures in a glass test tube immersed in hot water for 5, 10, 15, and 30 min. at 80, 90, and 100°C each. The nematodes were freed from the gall with sharply pointed forceps after the gall was placed into water for 24 hr. All treatments were replicated 6 times. Fresh galls contained an average of 630 nematodes, of which 82% were alive; five-year-old galls had 694 nematodes, of which 72% survived. The survival of nematodes in galls that were heat-treated at 80°C for 30 min. was 90% in fresh galls and reduced to 68% in 5 year old galls. Fresh galls exposed to 90°C for 30 min. survival was 68% and only 10% in five year old galls. All of the individuals were killed in five year old galls that were treated at 100°C for 15 min.; however, there was 35% survival in fresh galls. These experiments demonstrate that *Anguina agrostis* has remarkable heat tolerance that gradually declines as it ages.

Host range determination of *Synchytrium solstitiale*: Issues as a candidate for biological control of yellow starthistle

F. M. ESKANDARI (1), W. L. Bruckart (1), T. L. Widmer (1)
(1) USDA, ARS, FDWSRU, Ft. Detrick, MD, USA
Phytopathology 100:S206

Synchytrium solstitiale, a chytrid recently evaluated for biological control of yellow starthistle (YST, *Centaurea solstitialis*), was found to cause infections on seedlings of commercially-important safflower (*Carthamus tinctorius*) and two North American natives, *Centaurea americana* and *C. rothrockii* (10%, 10%, and 25% incidence, respectively) when cotyledons were exposed to zoospores released in water. This compared to >90% incidence for inoculated YST seedlings in that same study. The object of the present study was to confirm susceptibility of safflower to *S. solstitiale*. Surface-sterilized leaves of YST (as susceptible control), safflower, Russian knapweed (*Rhaponticum* [*Acroptilon*] *repens*), and common crupina (*Crupina vulgaris*), were floated on sterile water in large (15 cm) glass Petri dishes containing zoospores. Five pieces of surface-sterilized galled leaf tissue were placed at four locations around the perimeter and at the center of each dish to give uniform distribution of zoospores in each test. Leaves of each test species were paired with YST leaves in each dish, thus providing uniform inoculum for each test species and YST within a dish. At least three dishes were set up for tests. Results confirm that both YST and safflower are susceptible to *S. solstitiale* and that Russian knapweed and common crupina are not. Data from both studies suggest differential susceptibility to *S. solstitiale* occurs among plants within the Asteraceae. Before proposal is made to introduce *S. solstitiale* for biological control of YST in the U.S., additional data will be needed concerning potential risk associated with such action, particularly relating to safflower and safflower culture in California.

Phytophthora pini Leonian, a valid and distinct species

M. GALLEGLY (2), C. Hong (1), P. Richardson (1), P. Kong (1)
(1) Virginia Tech, Virginia Beach, VA; (2) West Virginia University, Morgantown, WV
Phytopathology 100:S207

Leonian described *Phytophthora pini* in 1925. His one culture, isolated from roots of red pine growing in Minnesota, was essentially ignored as a species until 1956 when Waterhouse included it in her compilation of original descriptions. Later, in 1963, Waterhouse considered *P. pini* to be invalid because it was morphologically identical to *P. citricola* described by Sawada in 1927. We secured the ex-type and ex-authentic cultures of *P. citricola*, *P. pini*, and Chester's *P. cactorum* var. *applanata* and compared them morphologically and molecularly with each other and with isolates of *P. plurivora* Jung and Burgess (same as Gallegly and Hong's *P. citricola* II), *P. citricola* I and *P. citricola* III. The results show that *P. pini* is identical to *P. citricola* I in all molecular and morphological characters and different from *P. plurivora*, the ex-type *P. citricola*, and *P. citricola* III. Incidentally, the ex-type culture of *P. cactorum* var. *applanata* is identical to isolates of *P. plurivora*. Thus, *Phytophthora pini* is resurrected to distinct species status.

Characterization of _Pythium_ species causing Pythium blight on snap beans in the eastern US

L. HARRISON (1), S. L. Rideout (1)
(1) Virginia Tech, Painter, VA, USA
Phytopathology 100:S207

The Eastern Shore of Virginia (ESV) is an important snap bean (*Phaseolus vulgaris* L.) growing region, but profitable yields are threatened by Pythium blight, one of the most severe snap bean diseases in the US. Although this disease is well documented, the species of *Pythium* causing this disease have not been well characterized. This information is important for determining management strategies. Isolates were collected to establish the causal agent(s) of Pythium blight on snap beans. Because of the pathogen's wide host range and distribution, isolates were recovered from different hosts, including other legumes, cucurbits and solanaceous crops, and from multiple snap bean-growing areas in the ESV, Georgia, and New Jersey. 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 each isolate was characterized by morphology and sequence analysis of the rDNA-internal transcribed spacer (ITS) regions. All ESV isolates were identified as *Pythium aphanidermatum*, except for one *Pythium myriotylum* and one *Pythium ultimum* isolate. Both *P. aphanidermatum* and *P. ultimum* were recovered from New Jersey crops. *P. aphanidermatum* was also isolated from symptomatic plants in Georgia; however, multiple Georgia isolates had 99-100% ITS sequence similarity with *Pythium deliense* Meurs accessions in GenBank. These isolates also had *P. deliense*-characteristic morphology, producing oospores measuring 16.5 µm diameter and similar, but less inflated, sporangia than *P. aphanidermatum*. Putative *P. deliense* isolates will be further characterized by sequence analysis of the cytochrome oxidase II gene. *P. deliense* has not yet been reported on common bean. This research verifies that multiple *Pythium* spp. are responsible for Pythium blight symptoms on snap beans.

Does a _Vicia villosa_ cover crop induce general suppression of Fusarium wilt of watermelon?

J. HIMMELSTEIN (2), K. Everts (2), J. Maul (1)
(1) Sustainable Agriculture Systems Laboratory, USDA-ARS, Beltsville, MD, USA; (2) University of Maryland, College Park, MD, USA
Phytopathology 100:S207

Current triploid watermelon cultivars have little resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (FON) and yield losses are increasing in the eastern U.S. A *Vicia villosa* (hairy vetch) green manure, suppressed watermelon Fusarium wilt in previous trials, but the mechanism of this suppression is unknown. The objective of this experiment was to determine if the *V. villosa* cover crop suppresses Fusarium wilt via general suppression by looking at the rate of soil respiration and microbial activity in the presence of three cover crops at two locations in Maryland. Fall planted *V. villosa*, *Trifolium incarnatum* (crimson clover), and *Secale cereale* (rye) were grown and incorporated as green manures into the soil prior to planting the watermelon. FON was applied three days after transplanting at the Salisbury location and five days after transplanting in Beltsville. No visible wilt symptoms were observed at either location. Hairy vetch and crimson clover caused a significant increase in total fruit yield at Beltsville. The respiration data revealed that microbial activity increased significantly directly after cover crop incorporation, then dropped down to pre incorporation levels for 3 months. As the watermelon matured the respiration rate increased once again. Crimson clover and hairy vetch plots had similar respiration rates and we are testing for correlations between cover crop induced changes in soil respiration and disease suppressiveness.

Multianalyte immunohistochemical investigation of relative hormone levels in potato purple top phytoplasma-infected tomato plants

Y. JIANG (1), W. Wei (1), Y. Ding (1), W. Wu (1), R. E. Davis (1), R. W. Hammond (1), Y. Zhao (1)
(1) Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD
Phytopathology 100:S207

Phytoplasmas are small, cell wall-less bacteria responsible for numerous diseases in agriculturally and environmentally important plant species worldwide. Phytoplasma infections of plants induce symptoms including excessive shoot proliferation, witches'-broom growths, general stunting, rapid senescence (yellowing), and abnormal floral development (virescence and phyllody). These symptoms indicate that hormonal balance may be disrupted in affected plants. The lack of plant hormone biosynthesis genes in all completely-sequenced phytoplasma genomes implies that the presumed hormonal imbalance in phytoplasma-infected plants may be caused either by changes in endogenous hormone levels or by alterations in sensitivity to hormones. The present study was aimed at understanding the mechanism underlying phytoplasma-induced host hormonal imbalance. Tissue sections prepared from Columbia Basin potato purple top (PPT) phytoplasma-infected and healthy tomato plants were subjected to comparative immunohistochemical analyses using antibodies against auxin (IAA), cytokinins (6-BA, trans-zeatin riboside, and cis-zeatin riboside), abscisic acid (ABA), and gibberellic acid (GA3). The results revealed notable changes in levels of plant hormones in PPT-infected vs healthy plants. Findings from the study will aid understanding of the roles of plant hormones in phytoplasma pathogenesis and disease symptom expression.

***Phytophthora phaseoli*; destroyer of lima bean production**

S. G. KUNJETI (1), N. M. Donofrio (1), A. G. Marsh (1), B. C. Meyers (1), T. A. Evans (1)
(1) University of Delaware
Phytopathology 100:S207

Phytophthora phaseoli Thaxt., an oomycete plant pathogen and close relative to *Phytophthora infestans* (Blair et al. 2008), causes downy mildew of lima bean (*Phaseolus lunatus* L.) during cool and humid weather conditions. Since its first report in 1889, the vegetable processing industry of the humid eastern US has been negatively affected by this disease. Since 1889 *P. phaseoli* has evolved six races A,B,C,D,E and currently prevalent race F in lima bean fields. Developing lima bean cultivars with durable resistance to this pathogen is a more environmentally friendly and cost-efficient method of disease management than pesticide application. To develop such a cultivar, it is necessary to understand the underlying mechanism of how the pathogen breaks down the plant's defenses. To date, nothing is known about the molecular interactions that occur during this plant-pathogen interaction. Towards a better understanding of these mechanisms, we used next generation sequencing technology (Illumina) to compare global gene expression of plate-grown and plant-grown *P. phaseoli*. Our computational analysis of the transcripts showed that most of the effector genes that were over-expressed in *P. infestans* while infecting potato leaf tissue were also over-expressed in *P. phaseoli* while infecting lima bean hypocotyls. Some of the well-characterized effectors like INF1, and INF4 were confirmed by performing RT-PCR using plate-grown, plant-grown mycelium and lima bean pods infected with *P. phaseoli* as template. Effector genes that were expressed in *P. phaseoli* when infecting lima bean pods were consistent with the genes expressed when infecting lima bean hypocotyls. Our results suggest that like in *P. infestans* infection of potato, *P. phaseoli* requires the same effector genes for the infection of lima bean pods and hypocotyls.

Comparison of the detection of _Xanthomonas campestris_ pv. _campestris_ in Brassica seeds using agar plating and real-time PCR

A. MARQUES (2), S. Abramova (1), A. Ignatov (1), A. Sechler (3), N. Schaad (3)
(1) All-Russian Institute of Phytopathology; (2) Embrapa Recursos Genéticos e Biotecnologia; (3) FDWSRU, USDA-ARS
Phytopathology 100:S207

Black rot of Crucifers, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is a serious seedborne disease worldwide. Although several assays are available for detection of Xcc from seed, seeds remain the major source of inoculum. We compared the recovery of Xcc on three media, mFS, NSCAA, and mCS20ABN from spiked and naturally infested seed lots using the industry standard seed testing protocol. The highest recovery of Xcc was found to be equal in spiked seed extracts on mFS and NSCAA. Recovery of Xcc from naturally infested seed was found to be higher on mFS (100 cells/ml) than NSCAA (30 cells/ml). Media mCS20ABN (Chang et al., 1991) was found to be frequently overgrown by non-target bacteria making identification difficult. For PCR we adapted the hrpF PCR primers of Berg et al. (2005) for RT-PCR by designing a probe sequence between primers DLH120 and DLH125. The resulting primer and probe set was optimized,

tested, and used for the confirmation of suspect Xcc colonies from agar media, and detection of Xcc from the seed soak. This adapted RT-PCR primer and probe set was found to react with Xcc and other Xanthomonas crucifer pathogens, including *X. raphani*, *X. armoraciae*, *X. barbarae*, and *X. aberrans*. The sensitivity of the primer and probe set was 500 cells per milliliter. Direct RT-PCR of the spiked seed soak was not reliable; detection was possible only from DNA extractions of the spiked seed soak.

Use of isoparaffin-based oil for controlling dollar spot and gray leaf spot in turfgrasses

D. S. MCCALL (1), A. E. Nichols (1), M. A. Cutulle (1)
(1) Virginia Tech, Blacksburg, VA, USA
Phytopathology 100:S208

Civitas fungicide is an isoparaffin-based oil, reported to trigger Induced Systemic Resistance (ISR) within plants. Little is known about how isoparaffin-based oils control turf diseases. Field trials were conducted on perennial ryegrass (*Lolium perenne*) and creeping bentgrass (*Agrostis stolonifera*) for control of gray leaf spot and dollar spot, respectively. Civitas (45.2 liters mineral oil per ha) plus Civitas Harmonizer (2.86 liters proprietary pigment dispersion per ha) was applied alone and in mixture with common fungicides for controlling each disease. Disease was assessed by visually estimating percentage of plots showing symptoms. The isoparaffin oil plus pigment has consistently reduced each disease tested when compared with untreated checks. Tank mixture of the oil plus pigment combination with reduced rates of thiophanate methyl (3336 Plus) controlled gray leaf spot as well as full rates of thiophanate-methyl. These results indicate that this isoparaffin oil plus pigment product suppressed disease during mild outbreaks of dollar spot and gray leaf spot, and can be mixed with reduced rates of several common fungicides during more severe outbreaks.

Clusters of defense-related genes in the genome of *Arabidopsis thaliana*

O. A. POSTNIKOVA (2), L. G. Nemchinov (2), A. M. Boutanaev (1)
(1) Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, Russia; (2) USDA/ARS, Plant Sciences Institute, Beltsville, MD, USA
Phytopathology 100:S208

Functional and physical clustering of unrelated genes known as operons is a characteristic of prokaryotic genomes. A concept and consequences of gene clusters in eukaryotic genomes are largely unexplored. In this work, we performed computer-generated analysis of the chromosomal distribution of genes associated with defense response in *Arabidopsis thaliana*. This analysis revealed numerous clustered genes whose co-regulation may be related to the defense responses. The genes were distributed among all chromosomes of *A. thaliana*. To support computer data, we arbitrarily selected two clusters and analyzed expression levels of their gene-members in Arabidopsis ecotypes Col-0 and C24 during infection with yellow strain of Cucumber mosaic virus (CMV(Y). Ecotype Col-0 is susceptible to CMV(Y), whereas C24 contains a dominant resistance gene RCY1. Our data showed that genes compiling two clusters were activated only in resistant ecotype C24. This indicated that co-regulation of neighboring, defense-related genes in the genome of Arabidopsis is strongly affected not only by their chromosomal location, but also by the basic mechanisms of genetic resistance to pathogens.

Isolates of *Fusarium graminearum* collected 40 to 300 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins

D. G. SCHMALE (1), T. Fetters (1), S. Ross (2), P. Tallapragada (2), B. Dingus (1)
(1) Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA; (2) Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA
Phytopathology 100:S208

The genus *Fusarium* contains important plant and animal pathogens, some of which produce dangerous secondary metabolites (mycotoxins). Many fusaria use the atmosphere to travel from one habitat to another, yet their atmospheric transport is poorly understood. We used autonomous (self-controlling) unmanned aerial vehicles (UAVs) to collect fusaria tens to hundreds of meters above ground level (AGL) at Virginia Tech's Kentland Farm in Blacksburg, VA. Eleven single-spored isolates of *Fusarium graminearum* collected with UAVs 40 to 300 meters AGL during fall, winter, spring, and summer months were able to cause Fusarium head blight on a susceptible cultivar of spring wheat and produce trichothecene mycotoxins. Nine of these isolates produced the mycotoxins deoxynivalenol (DON)/15-acetyl-DON, one isolate produced DON/3-acteyl-ADON, and one isolate produced nivalenol (NIV). To our knowledge, this is the first report of a NIV-producing isolate of *F. graminearum* in Virginia, and isolates producing DON/3-acetyl-DON are rare in populations of the fungus recovered from infected wheat plants in the eastern U.S. A new framework for understanding punctuated changes in the population structure of atmospheric fusaria based on the concepts of atmospheric transport barriers (ATBs) and Langrangian coherent structures (LCS) is being developed and tested at both local and regional scales. This work aims to transform our knowledge of the atmospheric transport of microorganisms and develop new paradigms that link field and atmospheric populations of toxigenic fusaria.

Detection of *Phytophthora ramorum* chlamydospores in soil by baiting and dilution plating

P. W. TOOLEY (1), M. M. Carras (1)
(1) USDA-ARS, Ft. Detrick, MD
Phytopathology 100:S208

Chlamydospores of *P. ramorum* produced by mixing 20 percent V8 juice broth cultures with sand and incubating over a 1 month period were used to infest field soil at densities ranging from 0.2 to 42 chlamydospores/cc soil. Chlamydospore recovery was determined by baiting with rhododendron leaf discs and dilution plating both when soil infestation was performed (time 0) and following 30 days storage at 4°C, as recommended in the soil and growing medium sampling protocol on the APHIS website (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml). Baiting was slightly more sensitive than dilution plating at time 0, allowing detection of *P. ramorum* down to 0.2 chlamydospores/cc soil compared with 1 chlamydospore/cc for dilution plating. Following 30 days of infested soil storage at 4°C, *P. ramorum* was detected using both methods at significantly ($P = 0.05$) higher levels than at time 0. The results indicate that storage of *P. ramorum*-infested soil at 4°C for 30 days can enhance recovery of the pathogen.

Increasing atmospheric carbon dioxide amplifies *Alternaria alternata* sporulation and antigen production, but does not impact sporulation of *Cladosporium phlei*

J. WOLF (3), N. R. O'Neill (2), C. A. Rogers (4), M. M. Muilenberg (4), L. H. Ziska (1)
(1) USDA-ARS, Crop Systems and Global Change Lab, Beltsville, MD, USA; (2) USDA-ARS, Systematic Mycology and Microbiology Lab, Beltsville, MD, USA; (3) University of Maryland, College Park, MD, USA; (4) University of Massachusetts School of Public Health, Amherst, MA, USA
Phytopathology 100:S208

Although the positive impact of elevated atmospheric carbon dioxide on pollen production has been established, impacts on fungal sporulation and antigen production have not been elucidated. This study examines the effects of rising atmospheric carbon dioxide on the quantity and quality of spores produced by fungi growing on timothy hay. Timothy grass (*Phleum pratense*) was grown at recent and projected future levels of carbon dioxide (300, 400, 500 and 600 μmol mol^{-1}). Leaves were used as substrate for the growth of *Alternaria alternata* and *Cladosporium phlei*. The abundance of spores produced by both fungi, as well as the size (microscopy) and antigenic protein content (ELISA) for *A. alternata*, were quantified. Timothy grass leaf dry weight and carbon-to-nitrogen ratio both increased at higher carbon dioxide levels. Leaf carbon-to-nitrogen ratio was positively correlated with the log of *A. alternata* spores produced per gram of leaf, but negatively correlated with antigenic protein content per spore. At the two highest levels of carbon dioxide, *A. alternata* produced nearly three-fold more spores and more than twice the total antigen per plant. *C. phlei* spore abundance increased with leaf carbon-to-nitrogen ratio, but overall spore numbers were much lower and per-plant production did not vary with carbon dioxide level. Elevated carbon dioxide often increases the biomass and carbon-to-nitrogen ratio of plant leaves. This study demonstrates that leaf changes induced by increasing carbon dioxide greatly enhance spore production by *A. alternata*, a ubiquitous allergenic fungus. This response may contribute to the increasing prevalence of allergies and asthma. Sporulation of *C. phlei*, a specialized pathogen of timothy grass, did not respond to increasing carbon dioxide in this study, suggesting that specialized and generalist fungal species may respond differently to rising atmospheric carbon dioxide. More study is needed to predict responses of different fungal groups to global changes.

Salicylic acid preconditioning increases tomato resistance to infection by potato purple top phytoplasma

W. WU (1), W. Wei (1), R. E. Davis (1), I. Lee (1), Y. Zhao (1)
(1) Molecular Plant Pathology Laboratory, ARS-USDA, Beltsville, MD
Phytopathology 100:S208

Columbia Basin potato purple top (PPT) phytoplasma is a newly discovered pathogen that causes serious diseases in potato and has the potential to affect other vegetable crops. Since the insect vector of PPT phytoplasma, the beet leafhopper, is a polyphagous species and has a wide geographic distribution, diseases associated with PPT phytoplasma infections may spread rapidly. The current study was aimed at investigating strategies to increase natural resistance of crops to PPT phytoplasma infections. The expression profiles of

a set of defense/pathogenesis-related genes were examined in PPT phytoplasma-infected tomato plants. Results indicated that a delayed onset and a lack of sustained expression of a subset of defense-related genes may be key factors involved in PPT phytoplasmal disease development. Pretreatment of plants with SA prior to graft inoculation with PPT phytoplasma significantly altered the expression patterns of the same subset of genes and resulted in partial resistance of tomato to PPT infection. The findings shed new light on molecular mechanisms of phytoplasma pathogenesis and should aid in devising new strategies to mitigate phytoplasmal diseases.

Analysis of visual symptomatology in peach and plum inoculated with U.S. *Plum pox virus* isolates

A. Younkins (1), A. L. Stone (3), D. J. SHERMAN (3), W. L. Schneider (3), R. Scorza (2), V. D. Damsteegt (3)
(1) Dickinson College, Carlisle, PA, USA; (2) USDA-ARS-AFRS, Kearneysville, WV, USA; (3) USDA-ARS-FDWSRU, Frederick, MD, USA
Phytopathology 100:S209

Plum pox potyvirus (PPV) is an economically devastating potyvirus that affects *Prunus* species. Discovered in the United States in 1999, the Pennsylvania PPV isolates were primarily found in peaches (*Prunus persica*). When several of these original Pennsylvania isolates were inoculated onto plums (*Prunus domestica*), the isolates either did not transmit or showed few symptoms. This suggests that Pennsylvania PPV isolates were more adapted to peach as a host. An expanded experiment was designed using a greater number of Pennsylvania and New York PPV isolates to identify a U.S. PPV isolate with severe visual symptoms in plums, and to determine if symptom severity correlated with PPV titer. Two plum varieties (Bluebyrd and Stanley) were inoculated with fourteen PPV isolates from New York and Pennsylvania by aphid (*Myzus persicae*) or by grafting. Visual symptom severity was determined using a standardized symptom rating system. PPV titers were measured using Enzyme Linked Immunosorbent Assay (ELISA) and Real-time one step reverse transcription-PCR (RT-PCR). In contrast to PPV infection in peach, there was little correlation between average symptom rating and average ELISA titer or average Real-time RT-PCR Ct value in plum. One PPV isolate had been maintained for 10 years in both hosts: peach and plum. When this isolate was inoculated onto plum from both peach and plum sources, differences in titer and symptoms showed a possible host adaptation between PPV maintained in plum or in peach tissue.

Author Index