

Tactical sciences for plant viruses to foster food security: the potato case

Prof. Sébastien Massart – Gembloux Agro-Bio Tech – Liege University



Dr. Kris De Jonghe – ILVO Plant Sciences



Belgian scientific plant health symposium

15/10/2020 - Online



INTERNATIONAL YEAR OF PLANT HEALTH 2020

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Food security and plant pathogens/pests in 2020



for a fair, healthy and environmentally-friendly food system



Healthy production
Healthy environment

Healthy processing
Healthy consumption
Healthy socio-economic relations



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Plant health in 2020, the covid-19 year

"Pay a little now or pay a lot more later"

Prevention is significantly less expensive than containing and eradicating an outbreak.







Pandemics may cause significant global economic slowdown.

The eradication of a disease can take several years and cost millions of dollars.

Acting before a crisis averts is a far more cost-effective approach.



Food and Agriculture Organization of the United Nations



International Plant Protection Convention



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Article <https://www.health.belgium.be/nl/news/de-overdracht-van-ziekten-bestrijden-overeenkomsten-tussen-mens-en-plant>

federal public service
HEALTH, FOOD CHAIN SAFETY
AND ENVIRONMENT

Plant health in 2020, the covid-19 year

Access to a safe and stable supply of food is now more crucial than ever.

While Covid-19 is affecting human health, plant pests and diseases continue to pose a threat to food security.

A threat to plant health is a threat to the health and prosperity of people, especially the most vulnerable.

Protecting plant health in an emergency



Protecting plants, protecting life!



Food and Agriculture Organization of the United Nations



International Plant Protection Convention



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Article <https://www.health.belgium.be/nl/news/de-overdracht-van-ziekten-bestrijden-overeenkomsten-tussen-mens-en-plant>

federal public service
HEALTH, FOOD CHAIN SAFETY
AND ENVIRONMENT

Plant Health threats

- Well-known established, yet continuous threats

Phytophthora infestans – late blight in potato

- New emerging threats

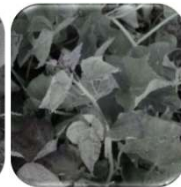
Changing conditions, changing food consumption patterns, etc.

'*Candidatus Liberibacter solanacearum*' - zebra chip

- Introduced threats

Globalisation & international trade

Sweet potato diseases



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Food safety & (bio)security

It starts at the ports of entry



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Photo credits:
favv;
landbouwleven,
keurpunt,
ILVO

Food safety & (bio)security

Phytosanitary risks by “uncontrolled” introductions

E-commerce as a pest pathway

- Internet is a convenient means for products to bypass the application of phytosanitary measures or scrutiny through PRA
- Few NPPOs used to factor this pathway into their risk analyses
- There is no effective mechanism for detecting products holding phytosanitary risks sold online



Seed/fertilizer/mulch mix



Wild flower seeds



Bulbs



Books infused with seeds

How to deal with this ??



Dirty camping gear & trekking clothes



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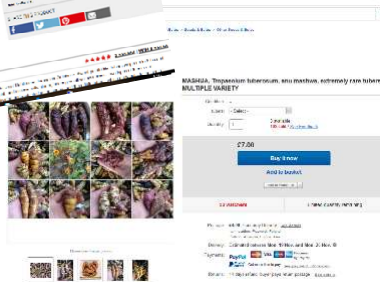
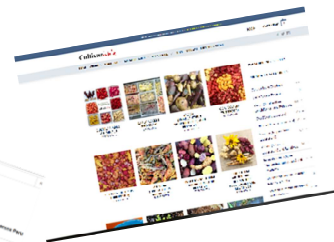
Food safety & (bio)security

Growing internet shop market shares

Buy Sweet Potatoes Online - Best Price -Faster Delivery ...
<https://onlinesabjiwala.com> | buy-sweet-potatoes-online • Vertaal deze pagina
 Buy Sweet Potatoes Online - Fresh Vegetables from the market - Faster Delivery - Lowest price ever - Healthy Benefits - Check the prices of Sweet Potatoes ...

Sweet Potato | Bhajiwala | Ecommerce Shop / Online ...
<https://www.indiamart.com> | Sweet Potatoes • Vertaal deze pagina
 Sweet Potato offered by Bhajiwala, a leading supplier of Sweet Potatoes in Kasarvadavai, Thane, Maharashtra. The Company is registered with IndiaMART ...

Shop for Sweet Potatoes & Yams for Fast Delivery | FreshDirect
<https://www.freshdirect.com> | browse | id=po_sweet • Vertaal deze pagina
 You can rely on FreshDirect for premium quality and friendly service.
 Ontbrekend: interesse!



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Food safety & (bio)security We are also to blame !



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Food safety & (bio)security EPPO campaign

How you can help

- Never ever illegally bring home plants, seeds, flowers, fruit and vegetables.
- If you do wish to import plants into your country, contact your local Plant Health authorities to find out what you can do legally.
- If you are carrying any plants, seeds, flowers, fruit and vegetables, declare them to the Customs official.



When travelling, avoid bringing back to your home plants or other plant items ... because plant pests or diseases may be hiding on them.



Pests and diseases can hide on plants. Please do not bring home plants, seeds, fruit, vegetables or flowers.

This leaflet was prepared by:
[your NPPO]

Help us to protect our crops and beautiful countryside against plant pests and diseases

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In collaboration with the European and Mediterranean Plant Protection Organization (OEPP/EPPO - www.eppo.org)

Food safety & (bio)security



Self-sustainable long-term network that supports coordination and collaboration in the area of phytosanitary research

NATURE PLANTS | VOL 6 | AUGUST 2020 | 902-905 | www.nature.com/natureplants

Science diplomacy for plant health

The battle between humans and plant pests is as old as agriculture, but the movement of pests as a consequence of human activities has been exacerbated only recently. International research collaboration is increasingly important to tackle pests causing serious damage to economies.

Baldissera Giovani, Sylvia Blümel, Ralf Lopian, David Teulon, Stephanie Bloem, Cristina Galeano Martínez Camilo Beltrán Montoya, Carlos Ramon Urias Morales, Sridhar Dharmapuri, Visoni Timote, Nico Horn, Mekki Chouibani, Jean Gérard Mezui M'Elia, Veronica Herrera, Aurélie Castinel, Con Goletos, Carina Moeller, Ian Naumann, Giuseppe Stancanelli, Stef Bronzwaer, Sara Tramontini, Philip MacDonald, Loren Matheson, Géraldine Anthoine, Kris De Jonghe, Martijn Schenk, Silke Steinmüller, Elena Rodriguez Maria Leonor Cruz, Jo Luck, Greg Fraser, Sarah Brunel, Mirko Montuori, Craig Fedchock, Elspeth Steel, Helen Grace Pennington, Roger Day, Jean Pierre Rossi and Jingyuan Xia

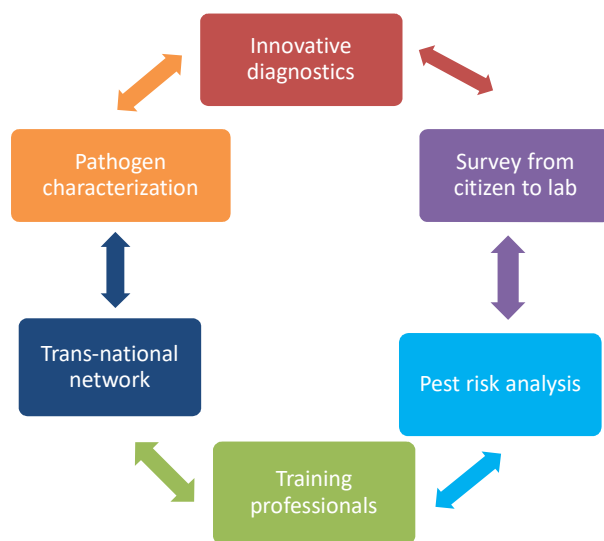
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Tactical sciences ?

Generating knowledge and information on how crises can emerge and evolve and how they can be anticipated, resolved or managed



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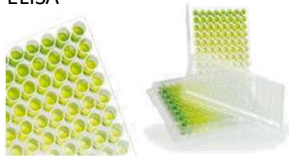


1. The rise of sequencing technologies

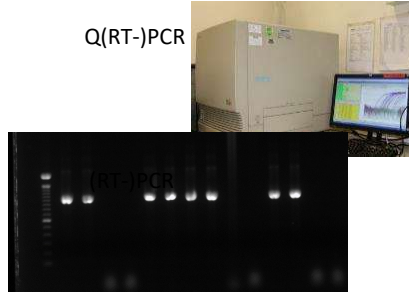
routine plant virus diagnostics

Innovative diagnostics Pathogen characterization

ELISA



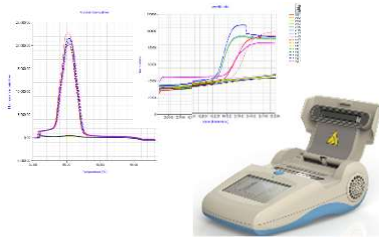
Q(RT)-PCR



Electron microscopy TEM - SEM



LAMP (Loop mediated isothermal amplification)



Mechanical inoculation on indicator plants



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1. The rise of sequencing technologies

Sequencing nucleic acids from 2000 to 2020 ?

Innovative diagnostics Pathogen characterization

90'ies

1.000

2020

1.700.000.000.000



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1. The rise of sequencing technologies

Innovative
diagnostics

Pathogen
characterization

Sequencing nucleic acids from 2000 to 2020 ?

**CLEARANCE
SALES :** **99.999 % OFF &
Better quality**



**Pay only 0.001 %
of original genome
price**

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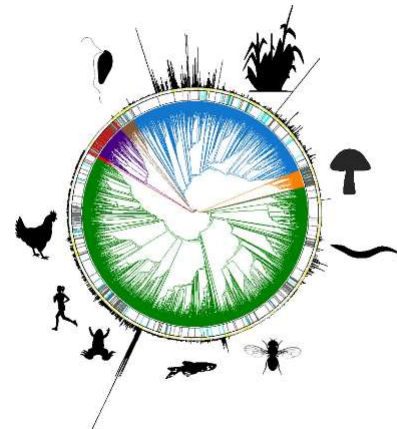
1. The rise of sequencing technologies

Innovative
diagnostics

Pathogen
characterization



What are the scientists doing?



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1. The rise of sequencing technologies

Innovative diagnostics Pathogen characterization

Genomes & Pathogens ?

Available online at www.sciencedirect.com
ScienceDirect **Current Opinion in Biotechnology**

Genomics of foodborne pathogens for microbial food safety
 Marc W Allard, Rebecca Bell, Christina M Ferreira, Narjol Gonzalez-Escalona, Maria Hoffmann, Tim Muruvanda, Andrea Ottesen, Padmini Ramachandran, Elizabeth Reed, Shashi Sharma, Eric Stevens, Ruth Timme, Jie Zheng and Eric W Brown

CMR **Whole-Genome Sequencing in Outbreak Analysis**
 Carol A. Gilchrist¹, Stephen D. Turner^{2*}, Margaret F. Riley^{3,4,5}, William A. Pesti, Jr.^{6,7}, Erik L. Hewlett^{8,9}
Department of Medicine, Public Health, Microbiology, and Pathology; School of Medicine, School of Law, and Biomechanical School of Dentistry and Public Health; University of Virginia, Charlottesville, Virginia, USA

frontiers in Bioengineering and Biotechnology **REVIEW**
 published: 15 May 2016
 doi: 10.3389/fbioe.2016.00104

Cyberbiosecurity Challenges of Pathogen Genome Databases
 Boris A. Vinatzer¹, Lenwood S. Heath², Hussain M. J. Almohri³, Michael J. Stulberg⁴, Christopher Lowe⁵ and Song Li^{6*}

¹School of Plant and Environmental Sciences, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, United States; ²Department of Computer Science, Virginia Polytechnic Institute and State University, Blacksburg, VA, United States; ³Department of Computer Science, Kuwait University, Kuwait City, Kuwait; ⁴Animal and Plant Health Inspection Service (APHA), Riverside Park, MD, United States; ⁵Biotechnology Research Center, Agricultural Research Service, USDA, Beltsville, MD, United States

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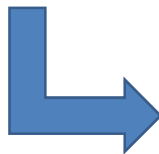
1. The rise of sequencing technologies

Innovative diagnostics Pathogen characterization

Genomes of known plant pests ?

Potato virus Y : - 460 full genomes (10 kb)
 - 3,300 sequences

Ralstonia solanacearum species complex
 - 102 full genomes (5.8 Mb)
 - 42,000 sequences



Virus Evolution, 2019, 5(2): vey037
 doi: 10.1093/ve/vey037
 Research article

Potato virus Y; the Andean connection

Segundo Fuentes,¹ Roger A.C. Jones,^{2,3} Hiroki Matsuoka,⁴ Kazusato Ohshima,⁴ Jan Kreuze,⁵ and Adrian J. Gibbs^{2,4,1}

Traceback the origin and timing of emergence of the different strains (O, N...)

Show that human activities have shaped the diversity of PVY

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1. The rise of sequencing technologies

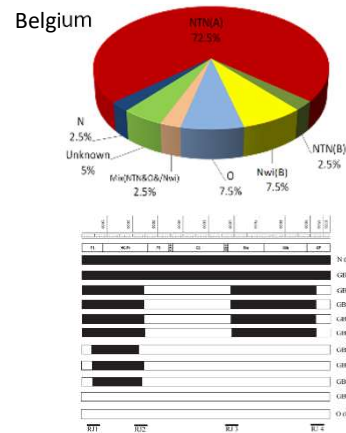
Innovative diagnostics Pathogen characterization

Genomes of known plant pests ?

Potato virus Y : - 460 full genomes
- 3,300 sequences

Belgium: Strain differentiation in support of seed potato certification

- Since 1984, first description of the mild PVY Wilga recombinant strain in Poland
- Since than, gradual increase in importance.
- Currently NTN and N-Wilga most important strains in Europa and Belgium



Source: Bahrami et al. 2014

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1. The rise of sequencing technologies

Innovative diagnostics Pathogen characterization



2016 – FAO-WHO

Genome sequencing is potentially a powerful tool in all relevant sectors in food, public health and animal/ **plant health**.

Strong relevance to a One Health approach.

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1. The rise of sequencing technologies

Innovative diagnostics Pathogen characterization

From Research to diagnostics :

2020 :

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N° 773139

Grant agreement N. 773139

DELIVERABLE N° 2.2

Title:

Guidelines for the selection, development, validation and routine use of high-throughput sequencing analysis in plant diagnostic laboratories

Valitest

Validation of diagnostic tests to support plant health

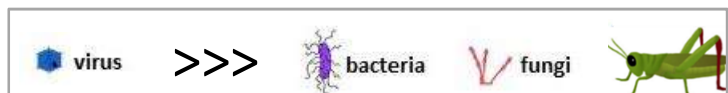
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1. The rise of sequencing technologies

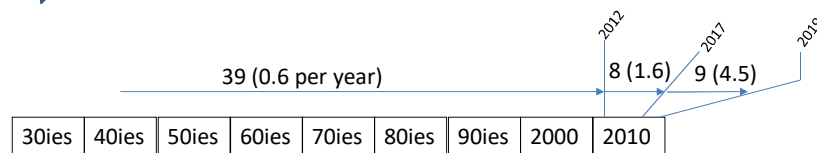
Innovative diagnostics Pathogen characterization

Discovery of new plant pests ?



Hundreds of new viruses discovered

Viruses and viroids on *Prunus* sp.



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2. Risks posed by new viruses ?

Pest Risk Analysis Pathogen characterization Trans-national network

Which one of the new plant viruses represents a risk for plant trade and production ?



PERSPECTIVE
published: 24 January 2017
doi: 10.3389/fmicb.2017.00045



A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies

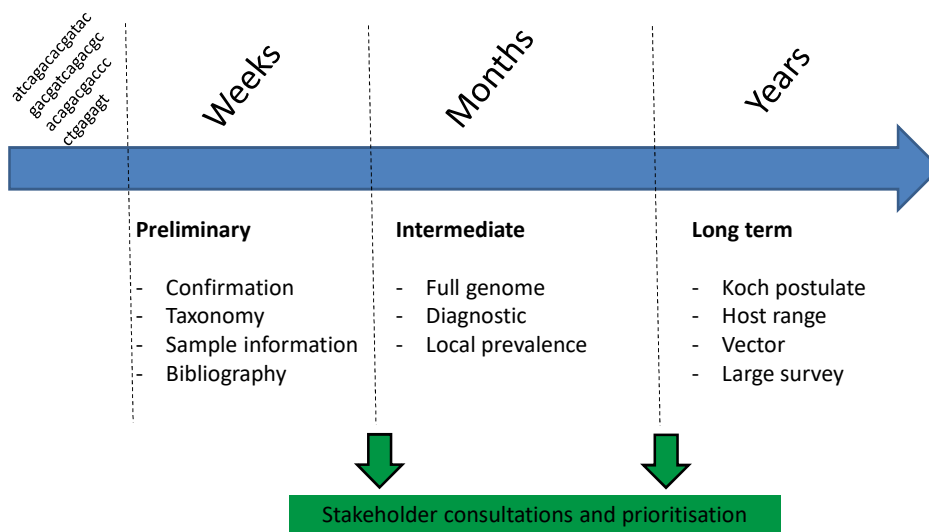
Sebastien Massart^{1*}, Thierry Candresse², José Gil³, Christophe Lacomme⁴, Lukas Predajna⁵, Maja Ravnikar⁶, Jean-Sébastien Reynard⁷, Artemis Rombou⁸, Pasquale Saldarelli⁹, Dijana Škorić¹⁰, Eeva J. Vainio¹¹, Jari P. T. Valkonen¹², Hervé Vanderschuren¹³, Christina Varveri¹⁴ and Thierry Wetzel¹⁵

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2. Risks posed by new viruses ?

Pest Risk Analysis Pathogen characterization Trans-national network



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2. Risks posed by new viruses ?

Pest Risk Analysis Pathogen characterization Trans-national network

Which one of the new plant viruses represents a risk for plant trade and production ?



Original Article Full Access

Biological and molecular characterization of Potato yellow blotch virus, a new species of the genus *Potyvirus*

C. Nisbet, W. A. Monger, S. Ross, R. F. Holmes, Y. Nova, C. Thomson, H. A. Goodfellow, C. Lacomme, C. J. Jeffries

First published: 08 September 2018 | <https://doi.org/10.1111/ppa.12943> | Citations: 4

- Discovery of a new potyvirus on breeding line of potato
- Host range of PYBV similar to PVA (solanaceous and indicator plants)
- Screening commercial seeds during 5 years: no detection

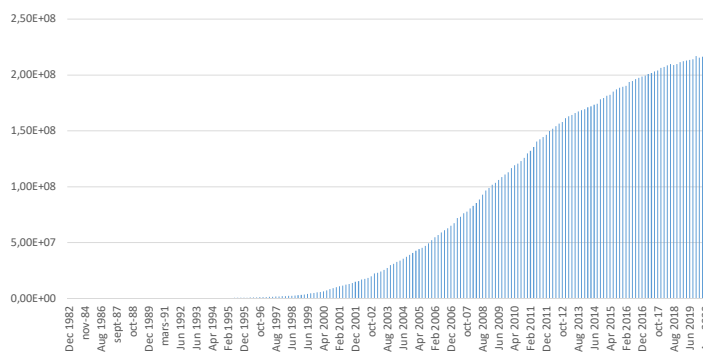
➔ Not the highest risk ?

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3. Bioinformaticians: the new stakeholder

Sequences available in Genbank database



1 week 2020

||

1982-1997

Innovative diagnostics
Pest Risk Analysis
Pathogen characterization
Trans-national network



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3. Bioinformaticians: the new stakeholder

Impact of data analysis on pest detection ?

Phytopathology • 2019 • 109:488-497 • <https://doi.org/10.1094/PHYTO-02-18-0067-R>

Virology

e-Xtra*



Virus Detection by High-Throughput Sequencing of Small RNAs: Large-Scale Performance Testing of Sequence Analysis Strategies

Sebastien Massart,[†] Michela Chiumenti, Kris De Jonghe, Rachel Glover, Annelies Haegeman, Igor Koloniuk, Petr Kominek, Jan Kreuze, Denis Kutnjak, Leonidas Lotos, François Maclot, Varvara Maliogka, Hans J. Marec, Thibaut Olivier, Antonio Olmos, Mikhail M. Pooggin, Jean-Sébastien Reynard, Ana B. Ruiz-García, Dana Safarova, Pierre H. H. Schneeberger, Noa Sela, Silvia Turco, Eeva J. Vainio, Eva Varallyay, Eric Verdin, Marcel Westenberg, Yves Brostaux, and Thierry Candresse



- A potato sample from Peru was sequenced and 2 viruses identified: PVX and a new nepovirus (not present in database and further identified as PVB)
- Two other samples: Grapevine and apple
- Identical datasets (10) sent to 13 laboratories (double blind test) -> application of bioinformatics tools to identify viruses present in the sample
- Worst case scenario (less sequences than expected – down to 50,000)

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3. Bioinformaticians: the new stakeholder

Impact of data analysis on pest detection ?

labID	Sensitivity			Average
	50,000	250,000	2,500,000	
A	10%	53%	90%	51%
B	30%	35%	80%	46%
C	60%	71%	80%	70%
D	50%	82%	100%	78%
E	30%	82%	80%	68%
F	80%	88%	100%	89%
G	20%	53%	100%	57%
H	30%	65%	70%	57%
J	70%	94%	100%	89%
K	40%	71%	90%	68%
M	50%	94%	90%	81%
N	30%	82%	90%	70%
O	20%	41%	40%	35%
P	20%	59%	70%	51%
R	100%	100%	100%	100%
S	50%	100%	100%	86%
T	90%	100%	100%	97%
V	60%	88%	80%	78%
W1	40%	82%	90%	73%
W2	60%	82%	90%	78%
X	30%	71%	80%	62%
AVERAGE	46%	75%	86%	70%

- 70 % sensitivity overall
- Huge differences between laboratories
- Sensitivity increased with sequencing depth and quantity of sequences from the viral species
- Potato viruses ?

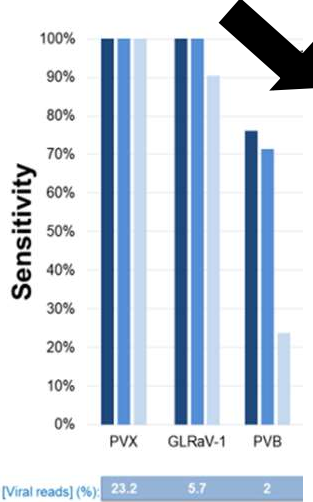


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3. Bioinformaticians: the new stakeholder

Impact of data analysis on pest detection ?



Ability to detect the virus was reduced for the unknown virus compared to PVX or GLRaV-1



Bioinformatics has a tremendous impact on virus detection

- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Trans-national network

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3. Bioinformaticians: the new stakeholder

Euphresco project (15 partners + 13 associated partners)
Coordination: A. Haegeman (ILVO)



The Plant Health Bioinformatic Network (PHBN)



- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Trans-national network



1. Develop training material
 2. Bioinformatics challenge on HTS data
 3. Data mining
- Seed for networking the new stakeholder



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3. Bioinformaticians: the new stakeholder

Guiding PRA by genome sequences ?

EPIDEMIOLOGY

Predicting reservoir hosts and arthropod vectors from evolutionary signatures in RNA virus genomes

Simon A. Babayan^{1,2}, Richard J. Orton³, Daniel G. Streicker^{1,3*}

Predicting the hosts and the vectors of mammalian viruses thanks to the genome sequence ?

Predictive accuracy : 72% for reservoir host & 99% for vector group



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3. Bioinformaticians: the new stakeholder

Guiding PRA by genome sequences

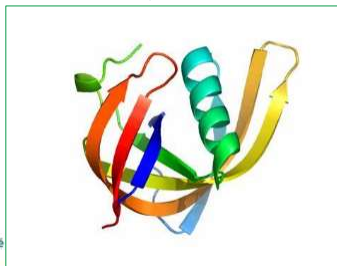
GENOPREDICT concept for plant viruses

TGAATGAGGATGAGGAAAAATGTCCATAC
GTGCTATGCCGCTTCCACTTCTCTGAGA
ACCTGCTTCTTGATTTCGTAGAA

Genomes

ILKYVCKTYFPASNREVYMKFLVTRVNTW
FCKFSRIDTFLLYKGVAHKSVDSEQFY

Proteins



Protein information = Modules

- 1) Function
- 2) Signature (amino acids)
- 3) 3-D conserved structures

+ transmission & host range



Genopredict dB



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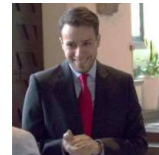
3. Bioinformaticians: the new stakeholder

Guiding PRA by genome sequences

What about plant viruses ?

- Data Mining & Machine learning algorithms
- Interesting results & variable depending on the biological properties
- Sensitivity >> specificity (risk of FP > FN)

PhD of Rachid Tahzima finishing & ongoing story



- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Trans-national network

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4. SEVIPLANT project

HTS as an untargeted diagnostic tool for virus screening

A case study for the identification of new harmful plant viruses in Solanaceae in Belgium



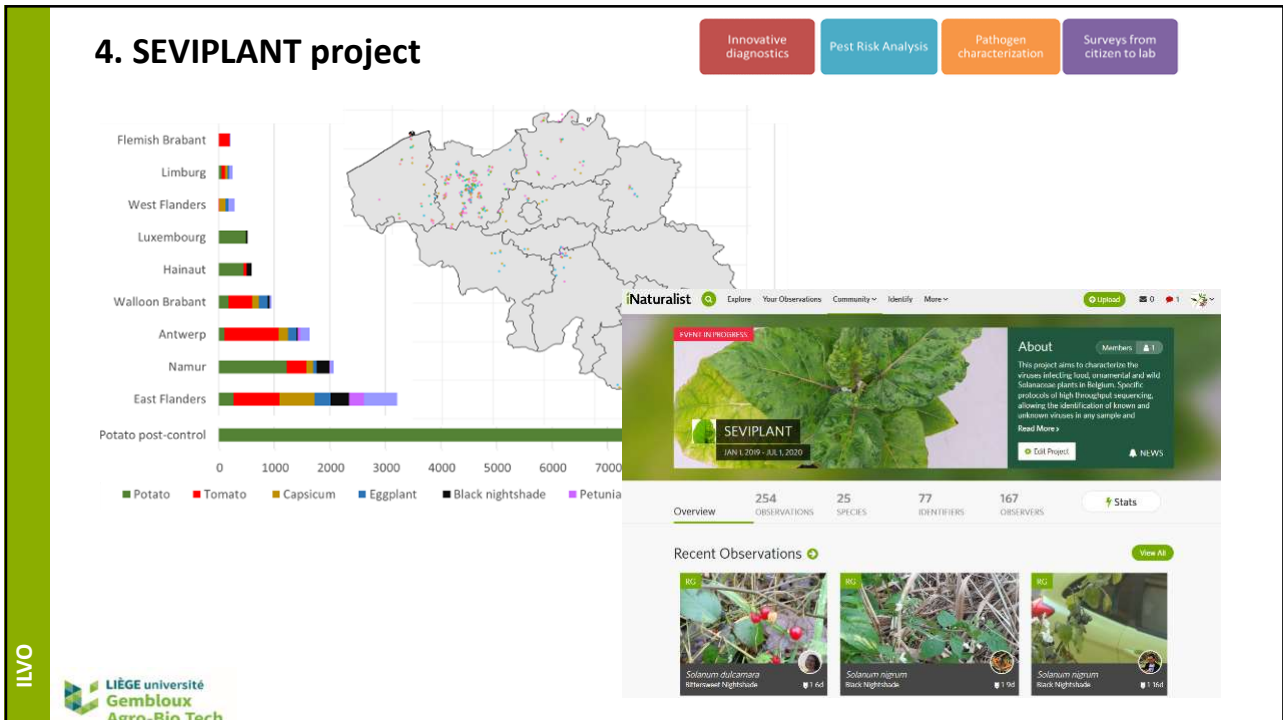
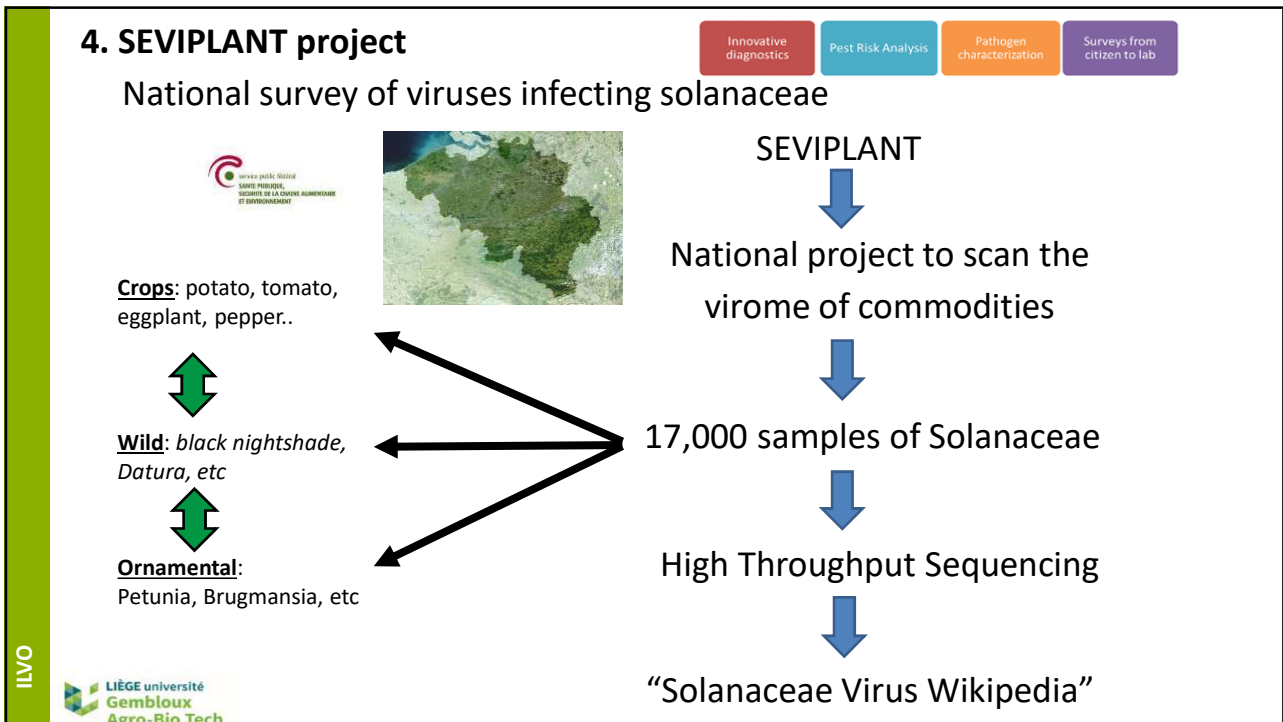
SEVIPLANT (RT 18/3)

- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Surveys from citizen to lab



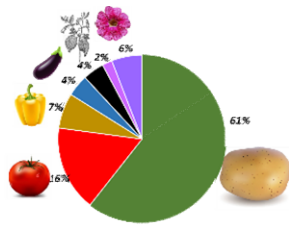
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4. SEVIPLANT project

- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Surveys from citizen to lab



- 1,300 samples sequenced
- 10 viral species detected
- 5 new species for Belgium
- New host for 3 viruses

Physostegia chlorotic mottle virus



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4. SEVIPLANT project

- Innovative diagnostics
- Pest Risk Analysis
- Pathogen characterization
- Surveys from citizen to lab

PhCMoV: informal EU network

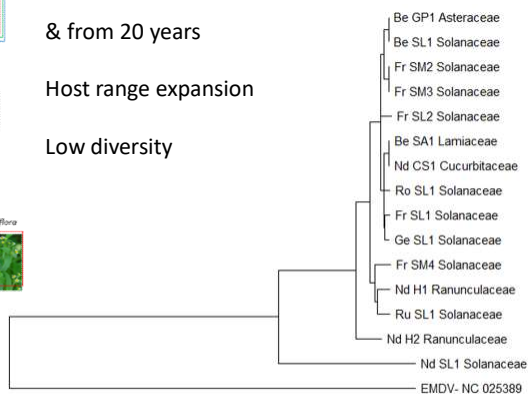


Present in many countries

& from 20 years

Host range expansion

Low diversity



0.10

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5. Surveys and status projects

Fyliber (RF14/6290) & VECTRACROP (2015 -D -168)



- Trans-national network
- Pest Risk Analysis
- Surveys from citizen to lab

Status of '*Ca. Liberibacter solanacearum*' and phytoplasma in carrot and potato



- Sampling in potato
- Sampling in carrot
- Sampling in both potato & carrot

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6. PRONC project

Phytosanitary risks of new crops in Belgian horticulture



National project (2 partners; ILVO & PCG)
Transnational project (7 partners)

PRONC – BE (2018-A-293)

- Trans-national network
- Pest Risk Analysis
- Pathogen characterization
- Surveys from citizen to lab

Increasing interest in growing and commercializing new crops, including some “forgotten” crops. => eg. tuber crops => **no info on phytosanitary status** & mostly vegetatively propagated

niche markets & “short chain circuits” - eg CSA farms

Examples: Yacon, ulluco, sweet potato, crosne, mashua, oca (new crops) and Jerusalem artichoke (forgotten crop)



RISK for our potato crops ?



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6. PRONC project

Trans-national network

Pest Risk Analysis

Pathogen characterization

Surveys from citizen to lab

Phytopsanitary risks of new crops in Belgian horticulture



National project (2 partners; ILVO & PCG)
Transnational project (7 partners)

PRONC – BE (2018-A-293)

Focus national project: **VIRUSES & NEMATODES**

- Where are these new crops cultured in Belgium, what are the varieties and how is the planting material distributed?
- What is the origin of planting materials of new crops?
- Which viruses and nematodes are associated with propagation materials and marketed new crops?
- What are the phytopsanitary risks of these organisms? → Biological characterization
- Which phytopsanitary measures can reduce the introduction and distribution of some of these new plant pathogens?

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7. Training professionals

Inspection services

- Sampling
- Identity and regulation
- Biology & epidemiology of diseases/pests

Symptomatology

Geographic distribution

Host range



Training professionals



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7. Training professionals

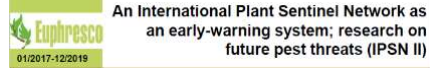
Botanical gardens and arboreta

- Trans-national network
- Pest Risk Analysis
- Surveys from citizen to lab
- Training professionals



Plant Health Charter - 2016	
<p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p>	<p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p>
<p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p>	<p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p> <p>Plant Health Charter - 2016</p>

The International Plant Sentinel Network (IPSN)



- Facilitate collaboration amongst institutes around the world,
- Focus on linking botanic gardens and arboreta, National Plant Protection Organisations (NPPOs) and plant health scientists.

The Belgian Plant Sentinel network (BE-PSN)

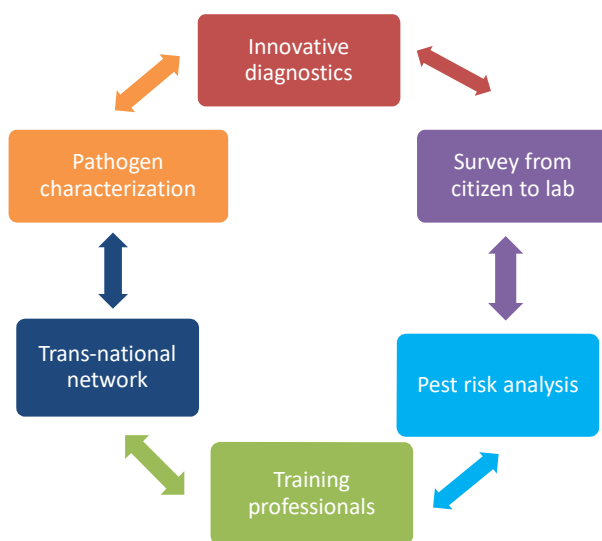


- Trainings & workshops
- Surveys
- Database

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Tactical sciences for plant viruses to foster food security



- ✓ Technological innovation brings new tools and generates new knowledge
- ✓ Integration of these tools and knowledge improve Plant Health management
- ✓ Optimizing resource utilisation
- ✓ International networks like Euphresco build synergies and accelerate diffusion of innovation
- ✓ Better food security through better plant health

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Thank you for your attention

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Dr. Kris De Jonghe – ILVO Plant Sciences

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(s. landbouw & visserij)