ABSTRACT BOOK

EPPO CONFERENCE ON DIAGNOSTICS OF PLANT PESTS

RECENT DEVELOPMENTS AND FUTURE TRENDS

AUSTRIAN AGENCY FOR HEALTH AND FOOD SAFETY -AGES

22-24 APRIL 2026

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Contents

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Welcome Message from the Organizing Committee

On behalf of the EPPO Secretariat and the Organizing Committee, we are looking forward to welcoming you to the EPPO Conference on Diagnostics of Plant Pests, taking place from 22 to 24 April 2026 at the Austrian Agency for Health and Food Safety (AGES) in Vienna, Austria.

This conference continues a long-standing and distinguished series of EPPO Conferences on Diagnostics, following previous conferences held in 1985, 1994, 2000, 2004, 2009, and 2015. Building on this strong tradition, the 2026 conference aims to provide a dynamic forum for sharing knowledge, fostering collaboration, and advancing the science and practice of plant pest diagnostics.

We are delighted that this Conference will bring together diagnosticians, scientists, inspectors, and other plant health stakeholders from across the EPPO region and beyond. Over the course of three days, participants will explore the latest developments in diagnostics, discuss emerging challenges, and exchange valuable experiences in the detection and identification of plant pests.

Early and reliable diagnosis is essential to protect plant health efficiently. In an increasingly interconnected world, strengthening our collective capacity in this field is more important than ever. We hope that this conference will contribute to enhancing cooperation, promoting innovation, sharing knowledge and supporting the harmonization of diagnostic approaches.

We hope that this Conference will provide you the opportunity to interact with colleagues, establish new connections, and enjoy the inspiring setting of Vienna.

We thank you in advance for your participation and hope you will have an engaging and productive Conference.

Organizing Committee

Organizing Committee

Scientific Committee

- Bart van de Vossenberg, the Netherlands Food and Consumer Product Safety Authority (NIVIP-NVWA), Netherlands
- Emilio Stefani, University of Modena and Reggio Emilia, Italy
- Heiko Ziebell, Julius Kuhn Institut (JKI), Germany
- Helga Reisenzein, The Austrian Agency for Health and Food Safety (AGES), Austria
- Johan Van Vaerenbergh, Institute for Agricultural and Fisheries Research (ILVO), Belgium
- Lee Robertson, National Center for Agricultural and Food Research and Technology (INIA-CSIC), Spain
- Maria Inácio, National Institute of Agricultural and Veterinary Research (INIAV), Portugal
- Pedro-Pablo Parra Giraldo, The French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France
- Piret van der Sman, Centre of Estonian Rural Research and Knowledge (METK), Estonia
- Tulin Sarigulertek, Ministry of Agriculture and Forestry, Türkiye

Local Organizing Committee at the Austrian Agency for Health and Food Safety (AGES)

- Dennis Bergamo
- Helga Reisenzein
- Philipp Hofer

EPPO Secretariat

- Anfisa Nikolaeva
- Charlotte Trontin
- Yassine Aoutil

Programme

Wednesday 22nd April 2026

08.00-09.00	Registration	
09.00-09.20	Welcome by Nico Horn (EPPO Officer-in-Charge)	
Session 1: Development and validation of tests Chair: Nico Horn (EPPO)		
9:20-9:25	Introduction Session 1	
9:25-10:10	K. Cardwell (OSU, US)	**Keynote presentation** Diagnostic Assay Validation Network (DAVN)
10:10-10:25	S. Bosman (NVWA, NL)	Expertise is a key component of fit-for-purpose validation and verification of tests
10:25-10:40	J. Foucher (GEVES, FR)	Detection of five different fungal taxa on flax seeds with one method
10:40-11:05	Break	
11:05-11:35	Welcome by Norbert Totschnig (Federal Minister of Agriculture and Forestry, Climate and Environmental Protection, Regions and Water Management, AT) and Johannes Pleiner-Duxneuner (AGES Managing Director)	
11:35-11:50	A. Benčič (NIB, SI)	Development of a multiprong real-time PCR test for the detection of the grapevine pathogen *Xylophilus ampelinus*
11:50-12:05	S. Loreti on behalf of V. Scala (CREA, IT)	The Italian Experience with *Pantoea stewartii* subsp. *stewartii*: Genomics, Diagnostics, and Insect-Mediated Transmission
12:05-12:20	B. Manser (Bioreba, CH)	Development and Validation of a real-time PCR kit for the detection of the *Ralstonia Solanacearum* Species Complex (RSSC)
12:20-12:35	M. Pilotti (CREA, IT)	Development and validation of real-time PCR for in-wood detection of *Ceratocystis ficicola*, the agent of canker, wood discolouration and wilt of common fig tree (*Ficus carica*)
12:35-12:50	B. Van De Vossenberg (NVWA, NL)	Development and validation of MUSTI; a High Throughput Sequencing test in support of fungal identification using rDNA and selected single copy barcodes
12:50-13:05	A. Vučurović (NIB, SI)	Establishing a validated Nanopore sequencing workflow for untargeted plant virus detection in diagnostic settings
13:05-14:05	Lunch	
Session 2: Advances in diagnostic technologies used in the field and in the laboratory Chair: Heiko Ziebell (JKI, DE)		
14:05-14:15	Introduction Session 2	
14:15-14:30	U. Persen (AGES, AT)	Can Scent Detection Dogs be Used for the Detection of *Erwinia amylovora* and *Cryphonectria parasitica*?
14:30-14:45	S. Kartakis (WUR, NL)	Assessing adoption drivers of a VOC sensor technology for pest and pathogen detection: Insights from a survey among European nurseries
14:45-15:00	J. Faria (INIAV, PT)	Early detection of the pinewood nematode based on volatile organic compounds (VOCs)
15:00-15:15	S. Van Der Linde (NVWA, NL)	MALDI-TOF mass spectrometry to advance the diagnostics of closely related *Fusarium* species
15:15-15:40	Break	
15:40-15:45	Welcome back	

15:45-16:00	N. Luchi (CNR, IT)	New molecular tools for early detection of forest pests and pathogens
16:00-16:15	A. Parle (DCU, IE)	Pushing the boundaries of in-field plant pathogen detection: Applying CRISPR-Cas technology for point-of-need testing for a resistant strain of *Phytophthora infestans*
16:15-16:30	A.B. Ruiz-Garcia (IVIA, ES)	Portable advanced molecular technologies for detection of plant pathogens
16:30-16:45	L. Laurenson (Fera, GB)	Can Nanopore Sequencing Transform Border Biosecurity Diagnostics?
16:45-17:00	E. Everaert (ILVO, BE)	Exploring Oxford Nanopore Sequencing Technology (ONT) Metabarcoding in Phloem Bacteria Diagnostics: Opportunities and Challenges
17:00-17:30	Break	
17:30-19:00	**Round tables**	

Thursday 23rd April 2026

08.30-09.00	Arrival	
09.00-09.25	Welcome and wrap up of round tables	
Session 3: Diagnostics of emerging & re-emerging pests Chair: Piret Van Der Sman (METK, EE)		
9:25-9:30	Introduction Session 3	
09:30-10:15	M. Sakalidis (DPIRD, AU)	**Keynote presentation** Species, what's in a name? The challenges of species identity in Plant Biosecurity and the use of diagnostics in practice. An Australian Perspective
10:15-10:30	G. Silva (NRI, GB)	Molecular diagnostic tests to strengthen seed systems
10:30-10:45	V. Gualandri (FMACH, IT)	Surveillance, symptomology, and status update of three economically emerging viruses in Trentino region in Northern Italy
10:45-11:45	Break + **Poster exhibition**	
11:45-11:50	Welcome back	
11:50-12:05	R. Gazis (UFL, US)	Strengthening Avocado Disease Surveillance through Enhanced Diagnostics for Scab and Laurel Wilt
12:05-12:20	C. Douanla-Meli (JKI, DE)	Development and validation of PCR tests to diagnose the new *Trichoderma* ear rot on maize
12:20-12:35	J. Reiterer (AGES, AT)	First report of *Verticillium dahliae* causing wilt of Pawpaw (*Asimina triloba*)
12:35-12:50	A. Minuto (CeRSAA, IT)	Bacterial Disease Outbreaks in Protected Crops and Nurseries: An Emerging Challenge
12:50-13:50	Lunch	
Session 4: Plant Health diagnostics from a broader perspective Bart Van De Vossenberg (NIVIP-NVWA, NL)		
13:50-14:00	Introduction Session 4	
14:00-14:15	K. Hughes (APHA, GB)	Development of diagnostics for inspectors
14:15-14:30	C. De Krom (NVWA, NL)	Applying different workflows in plant virus diagnostics
14:30-14:45	N. Mehle (NIB, SI)	From detection to risk assessment: Understanding the fate of plant viruses in organic waste fertilizers

14:45-15:00	A.S. Van Bruggen (NVWA, NL)	Challenges for diagnosis of tropical root-knot nematodes with a focus on *Meloidogyne enterolobii*
15:00-15:15	B. Vicelli (FMACH, IT)	Development of an early detection protocol of *Erwinia amylovora* from corbicular pollen to monitor its spread in apple orchards
15:15-15:30	T. Dreo on behalf of V. Grujić (NIB, SI)	Revealing diagnostic gaps and sequence diversity in *Xanthomonas* pathovars of common bean through molecular testing and DNA barcoding
15:30-16:30	Group picture + Break + **Poster exhibition**	
16:30-16:40	Welcome back + information to participants	
16:40-16:55	N. Pucci (CREA, IT)	Detection of *Xylella fastidiosa* in spiked dormant plant material at different growth stages, and in naturally infected olive trees with canopy vigour recovery
16:55-17:10	T. Dreo (NIB, SI)	From spiking to routine use: versatile applications of reference materials for *Xylella fastidiosa* diagnostics
17:10-17:25	A. Aspin (Fera, GB)	The National Collection of Plant Pathogenic Bacteria: The use of bacterial culture collections for the provision of reference standards: the cornerstone to accurate bacterial diagnosis
17:25-17:40	K. De Jonghe (ILVO, BE)	Validation of Nucleic Acid extraction internal control procedures for diagnosis
17:40-17:55	C. Pohn (AGES, AT)	Interplay of morphological and molecular methods in entomology
17:55-18:10	W. Menzel (DSMZ, DE)	Over 10 years' experience in potato virus proficiency tests as external proof of diagnostic laboratory competence
18:45	Departure of the bus from AGES to the social dinner	
19:15-23:15	**Social dinner**	

Friday 24th April 2026

08.00-08.30	Arrival	
08.30-08.35	Welcome	
Session 5: Initiatives for improving plant health cooperation in diagnostics Chair: Helga Reisenzein (AGES, AT)		
08.35-08.40	Introduction Session 5	
08:40-09:25	A.G. Moreira (IPPC)	**Keynote presentation** Beyond Standards: The IPPC's Approach to Strengthening Global Diagnostic Cooperation
09:25-09:40	L. Ferretti (CREA, IT)	The Italian phytosanitary laboratories network to improve and harmonize the diagnostics in plant health
09:40-09:55	F. Munaut (EC, BE)	European reference laboratories, a strong EU network supporting the plant health sector
09:55-10:10	M. Grossi De Sá (ANSES, FR)	EURL Nematodes: Collaboration to Strengthen and Harmonize Diagnostics
10:10-10:25	R. Vreeburg (NVWA, NL)	How the EURL Bacteriology supports international phytosanitary policy-makers on issues related to plant quarantine bacteria diagnostics
10:25-10:50	Break	
10:50-10:55	Welcome back	
10:55-11:10	H. Reisenzein (AGES, AT)	Bridging Expertise: The Power of Cross-Organisational Collaboration

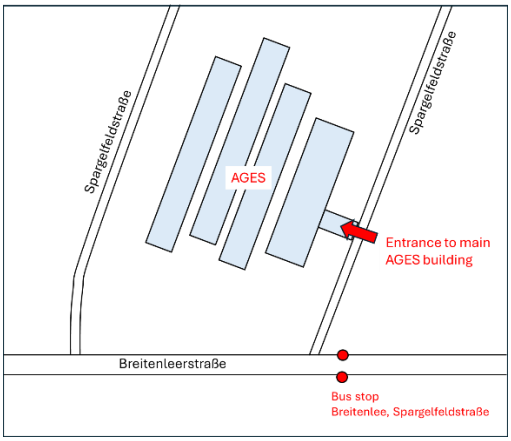
11:10-11:25	P.P. Parra Giraldo (ANSES, FR)	Towards a more reliable detection of quarantine *Elsinoe* species on citrus: Beyond sequences, primers and probes
11:25-11:40	A. Giesbers (NVWA, NL)	EURL Virology – advancing reliable diagnostics by bringing laboratories together
11:40-11:55	S. Rauch (ASTA, LU)	Strategic pest prioritization and diagnostic synergies across borders in Luxembourg
11:55-12:10	N. Denancé (GEVES, FR)	Towards Safer Seeds: The Changing Landscape of Seed Health Testing
12:10-12:30	Closing remarks	
12:30-13:15	**Cold lunch/departure**	
13:15	**Departure for social activities (optional)**	

Practical information

Meeting venue

Address:

AGES
1220 Vienna,
Spargelfeldstrasse 191
GPS coordinates:
48°15'08.9"N 16°29'00.6"E



Please note:

The route planner on Google Maps may direct you to the wrong AGES building (building complex near Rautenweg). The event will take place in the main AGES building (closer to Breitenleerstraße).
If you are travelling by public transport, take the 24A bus from Kagraner Platz and travel 6 stops to the 'Breitenlee, Spargelfeldstraße' stop. Cross Breitenleerstraße and walk along Spargelfeldstraße on the left-hand side for about 100 metres until you reach the pedestrian entrance to AGES.
If you search for 48°15'08.9"N 16°29'00.6"E on google maps, it will lead you to the right entrance.

Roundtables

Three round tables are organized on Wednesday 22nd of April. Please attend the round table that was attributed to you (check your badge)

Round table 1: Diagnostics for an increasing number of pests: what are the consequences for laboratories and how can this be managed to enhance the resilience of laboratories?

Moderators: Helga Reisenzein (AGES, AT) and Emilio Stephani (Unimore, IT)

Room: A/E.59

The continuously increasing trade of plants and plant products, the unceasing emergence of new pests, the changing climate supporting the spread of pests into new territories challenge the activity and organization of diagnostic laboratories. This round table will tackle the following questions:

- How can resources be used efficiently to meet the increased requirements?
- How do diagnosticians increase their expertise?
- How does the lack of reference material or specific information/test(s) for a robust detection/identification of pests impact diagnostics?
- What impact does the different phytosanitary categorization of organisms in different countries have on diagnostic laboratories?

Experts in diagnostics, laboratory managers and head of reference laboratories are warmly invited to contribute to this round table with their experience and solutions.

Round table 2: Emerging techniques for the detection of plant pests: Convenience, reliability and equivalence

Moderators: Pedro Pablo Parra (ANSES, FR) and Lee Robertson (INIA, ES)

Room: Lecture hall A/E.51

Advances in biotechnological tools have provided a plethora of methods to identify, detect and manage plant diseases. While there are valuable tools for the detection of plant pests, their application in diagnostic laboratories does not always respond to the necessity of having early, rapid and reliable results that allow effective controls. In this round table we aim to discuss the main criteria driving the selection of the method in relation to their pertinence, turnaround time, reliability and equivalence. Moreover, we would like to explore strategies and challenges for the adoption of emerging methods while maintaining the capabilities to perform more classical methods.

Round table 3: We need <u>YOU</u> to preserve expertise in classical diagnostic techniques

Moderators: Bart van de Vossenberg (NIVIP, NL) and Maria de Lurdes Inácio (INIAV, PT)

Room: A/E.56

This roundtable addresses the critical need for maintaining and developing expertise in classical diagnostic techniques (morphology, microscopy, culture-based methods) as many experienced taxonomists approach retirement. We will explore practical solutions including creating a "marketplace" of training opportunities where laboratories can share their expertise within and among institutions, and ways to make classical techniques more accessible to younger generations through modern tools. We aim to develop recommendations for funders and decision-makers on preserving this vital expertise for plant health diagnostics.

Keynotes Speakers

Kitty F. Cardwell
Founder, Microbe Finder, MiFi LLC; retired from the Institute of Biosecurity and Microbial Forensics, Oklahoma State University, United States of America

Dr Kitty Cardwell grew up in a small town in Texas. She obtained a bachelor's degree in Botany from the University of Texas and a PhD in Plant Pathology and Microbiology at Texas A&M University. She was a Peace Corps volunteer in Nicaragua and Colombia where she learned Spanish and gained field experience as a plant pathologist in national agricultural institutions. After graduate school, Kitty joined the International Institute of Tropical Agriculture (IITA) in Ibadan Nigeria where she was a research plant pathologist for 12 years. She returned to the U.S. in 2001 and became a National Program Leader in plant protection at the U.S. Department of Agriculture in Washington, DC. As a leader and grants administrator, she founded the National Plant Diagnostic Network and the Food and Defense Initiative, which continue to operate to this day. In 2016, Dr Cardwell was recruited to Oklahoma State University (OSU) where she became Professor and Director of the Institute of Biosecurity and Microbial Forensics (IBMF). IBMF is a center for innovation, research, and teaching in the scientific disciplines of biosecurity. While at IBMF, she drove the development of U.S. nation-wide project to build the Diagnostic Assay Validation Network. Kitty retired from OSU in 2023 but is still actively engaged in DAVN and she is the founding director of Microbe Finder (MiFi, Inc), a company that develops sequence-based diagnostic tests for plant, animal and human infectious diseases.

Monique Sakalidis
Department of Primary Industries and Regional Development & Harry Butler Institute, Murdoch University, Murdoch, Western Australia

Dr Sakalidis is a forest pathologist with over 20 years of experience across Australia, South Africa, Canada, and the United States, specialising in exotic, invasive, and emerging fungal and oomycete pathogens. During this time her research focused on how Anthropocene driven change influences pathogen behaviour, evolution, and disease risk across natural, urban, plantation, and agricultural systems, drawing on epidemiology, phylogenetics, and population genetics. As a Senior Research Scientist in the Plant Biosecurity Preparedness and Response team at the Western Australian Department of Primary Industries and Regional Development (DPIRD), Dr Sakalidis works to anticipate and mitigate emerging biosecurity threats across Western Australia. Applying strategic, science-based decision making, she leads and supports preparedness planning, develops tools and approaches to inform early decision-making during emergency responses, and provides authoritative advice during biosecurity incidents. Her work strengthens coordinated response capability and preparedness through collaboration with key stakeholders across government, industry, and academia.

Adriana Moreira
International Plant Protection Convention

Dr. Adriana G. Moreira is a charismatic leading expert in global phytosanitary policy and a Ph.D. Plant Pathologist (Virology) with over 17 years of experience. Currently serving as the Officer in Charge of the Integration & Support Team at the IPPC Secretariat (FAO), she oversees core operations, communications, and the coordination of international governing bodies. A key figure in standard-setting, Dr. Moreira has facilitated the adoption of over 60 International Standards for Phytosanitary Measures (ISPMs), diagnostic protocols, and including the successful adoption of the first in the IPPC's "next generation" of commodity standards. She is a prominent advocate for SDG2 (Zero Hunger), safe provision of humanitarian aid and gender equality within leadership and agricultural sectors. Her global impact has been recognized: she was named one of Forbes' "50 Women Taking Agriculture from Brazil to the World" (2024), a 2025 FAO-NSP "Optimistic Influencer in Building One Dynamic NSP," and an FAO Young-Employee awardee (2019). Dr. Moreira brings a unique blend of private sector, academic, and diplomatic expertise to the global plant health stage.

Keynote presentation 1

Diagnostic Assay Validation Network (DAVN)

Cardwell Kitty[1], Lapaire-Harmon Carrie[2], Groth-Helms Debi[3], Hope Amy [4], Luster Doug[5], Rivera Yazmínn[5], Sharma Poonam[1], Stack James[6]

[1]Institute of Biosecurity and Microbial Forensics, Oklahoma State University
[2]Plant Diagnostic Center, Department of Plant Pathology, University of Florida
[3]AgDia
[4]American Phytopathological Society
[5]Agricultural Research Service, United States Department of Agriculture
[6]Department of Plant Pathology, Kansas State University

The Diagnostic Assay Validation Network (DAVN) was developed to coordinate resources and activities related to plant diagnostic test development, validation, and use across the U.S. DAVN follows on from the EPPO Valitest project, with input from European counterparts. Grant funds from the U.S. Department of Agriculture (2020-2026) and partnership with the American Phytopathological Society, support the DAVN. Globally, there has been no consensus usage of diagnostic assay validation terminology nor of the underlying statistical foundation critical to accurate diagnostics. At times, the differences have been controversial. Clarity is critical to understanding and agreement among biosecurity practitioners, whether local, regional or international. In the U.S., during the early stages of DAVN, we assessed the resources available, existing infrastructure and what would be needed to promote robust diagnostic assay validation. The plant pathology innovation system in the U.S. was dispersed across universities, the private sector and the federal government. The science and language of diagnostic assay validation were not taught, and published information about diagnosing plant diseases was not organized. DAVN developed tools to assist and connect plant disease research scientists, diagnostic assay developers, validation research, and end users, to facilitate harmonization of protocols and technology transfer. We will demonstrate the language that has been adopted in the U.S., the framework for validation and statistical algorithms that are available to standardize test performance studies. The DAVN website has databases that organize information of published protocols in nucleic acid extraction and using controls in assays. There are webinars available, and guidelines for publishing research on assay development and validation. For a preview, please go to DAVN.

Keywords: Plant disease, diagnostic test, diagnostic assay, validation research.

Keynote presentation 2

Species, what's in a name? The challenges of species identity in Plant Biosecurity. An Australian Perspective

Sakalidis Monique L.[1,2], Katukuri Susheela[1], Lanoiselet Tamrika[1], Lanoiselet Vincent[1], Uloth[1], Marcus Visic Margaret[1], Kehoe Monica[1], Pain Nick[1], Wright Dominie[1], Maxwell Aaron[3], Christy Michelle[1]

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[3]Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra, ACT, 2601, Australia

Are species an artificial, anthropogenic construct or do they represent essential units of life, carrying meaning beyond our naming conventions? This is not a purely academic distinction, and from a biosecurity perspective a species name may distinguish a harmful organism from an innocuous one. Species names function as operational tools determining what is regulated, surveillance design, diagnostics validation, emergency response triggers and how pest absence is demonstrated. Yet species names are not static. They change as new data, technologies and interpretations emerge, creating challenges for those who must work with definitions that may be scientifically valid but operationally unstable. Australia's long geological isolation has shaped a unique, megadiverse biota that is highly vulnerable to invasive pests. The broad availability of suitable land and wide climatic range also favours an extensive domestic and export-driven agricultural sector. To safeguard these assets, Australia operates a dual biosecurity system with national measures led by the Commonwealth Department of Agriculture, Fisheries and Forestry, complemented by state and territory jurisdiction-specific border and regulatory controls. Using examples from Western Australia, which covers nearly one-third of Australia's landmass, we illustrate how fluid taxonomy affects operational biosecurity. Increasing pathogen incursions threaten the state's natural environment and $15.5 billion AUD of agricultural, fisheries and forestry exports, where detection and response efforts are challenged by vast distances, diverse flora and multiple climatic zones. Effective biosecurity relies on knowing whether an organism is present or absent and whether it poses a risk. When a new species is named solely on minor genetic or morphological differences, without understanding of its ecology, behaviour or impact, then effective risk assessment, test development, and regulatory decision-making become far more difficult. When species names change, impacts cascade across diagnostics, surveillance priorities, and policy decisions. Extending beyond laboratory and regulatory frameworks, names can also influence stakeholder engagement and public acceptance. This presentation explores how taxonomic uncertainty, diagnostic practice and risk communication intersect to shape biosecurity outcomes. We highlight how uncertainty undermines biosecurity and how well-characterised species enable robust policy, timely diagnostics and clearer stakeholder engagement, ultimately supporting better economic, environmental and biosecurity outcomes.

Keywords: species concepts, nomenclature, biosecurity, diagnostics, pest risk analysis.

Keynote presentation 3

Beyond Standards: The IPPC's Approach to Strengthening Global Diagnostic Cooperation

Moreira Adriana G., Martino Marina

International Plant Protection Convention

Effective and harmonized pest diagnostics are foundational to global plant health, enabling accurate and timely pest identification that supports safe international trade, strengthens food security, and protects the environment. However, the global diagnostic landscape remains fragmented, facing technical capacity gaps and a need for more harmonized and faster detection methods. To address these challenges, the International Plant Protection Convention (IPPC) facilitates a harmonized global approach. Under the IPPC, the Commission on Phytosanitary Measures (CPM) leads the development of International Standards for Phytosanitary Measures (ISPMs), which provide the global framework for countries to establish scientifically justified phytosanitary measures and are the baseline for international cooperation. A core component of this work is ISPM 27 (*Diagnostic protocols for regulated pests*)[1], which establishes the benchmark for official diagnostic procedures. Annexes to ISPM 27, the Diagnostic Protocols (DPs), provide internationally agreed methods for pest detection and identification, ensuring that diagnostic results are recognized across borders and for a wide-range of countries' capacities. They are developed by the Technical Panel on Diagnostic Protocols (TPDP) for specific pests or groups of pests and provide detailed guidance on their taxonomic status, biological and molecular characteristics, as well as the validated methods for their detection and identification. Accordingly, IPPC Diagnostic Protocols ensure robust and implementable standards for global plant pests diagnosis. In alignment with the IPPC Strategic Framework 2020-2030[2], the IPPC has identified diagnostic capacity building as a key development agenda item. Recognizing that standards alone are not enough to bridge the global capacity gap, the IPPC is moving toward a more integrated model: one that links early warning, standard setting, coordinated research, specialized training and an international network of diagnostic laboratory support. By integrating these strategic pillars, the IPPC aims to transform a fragmented landscape into a proactive, research-driven, and cohesive global diagnostic infrastructure. By encouraging cooperation among national and regional laboratories and integrating international standards such as ISPM 27, the IPPC seeks to advance diagnostic reliability and accessibility worldwide.

Keywords: IPPC, ISPM 27, Diagnostic Protocols, phytosanitary diagnostics, international cooperation.

[1] ISPM 27 (*Diagnostic protocols for regulated pests*): https://www.ippc.int/en/publications/593/
[2] IPPC Strategic Framework 2020-2030: https://www.ippc.int/en/strategic-objectives/ippc-strategic-framework/

Abstracts for presentations

Session 1: Development and validation of tests

Pr01

Session: Development and validation of tests

Expertise is a key component of fit-for-purpose validation and verification of tests

Bosman Saskia

The Netherlands Institute for Vectors, Invasive plants and Plant health (NVWA-NIVIP), the Netherlands

What is in a definition? Tests are defined as the application of a method to a specific pest and a specific matrix. But how can we ensure clear communication across disciplines on the definition of a test? The corner stone of reliable testing is validation and verification of tests. At NVWA-NIVIP different approaches to validation and verification are used based on the type of test, its intended use, and the circumstances of use. The common ground across the different disciplines is the role of the expertise in fit-for-purpose validation and verification of tests, which will be demonstrated with examples of validation and verification from morphological identification to high throughput sequencing. This means of test interpretation, validation, and verification allows the NVWA-NIVIP to comply with EU Official Controls Regulation, manage our accreditation and prepare to test for organisms that we have never encountered. This presentation will elaborate on the definition of test, validation, and verification and how the different disciplines at NVWA-NIVIP interpret this. Furthermore, I will show how NVWA-NIVIP implements fit-for purpose validation for different disciplines in our ‘fytoflex’ scope.

Keywords: Validation, Verification, Flexible scope, Expertise.

Pr02

Session: Development and validation of tests

Detection of five different fungal taxa on flax seeds with one method

Foucher Justine, Granon Claire, Le Guisquet Céline, Cesbron Gaël, Le Dare Lorine, Sérandat Isabelle, Malabarba Jaiana

French Variety and Seed Study and Control Group (GEVES), France

Flax (*Linum usitatissimum*) is an important crop cultivated for its seeds and fibre. Seeds are used to produce oil and the fibre is used in the textile industry. However, seed yield and quality can be affected by several fungi causing diseases such as Fusarium wilt, early blight, anthracnose, grey mould, and basal stem blight disease. These diseases, caused by *Fusarium* spp., *Alternaria linicola*, *Colletotrichum lini*, *Botrytis cinerea*, and *Boeremia exigua* var. *linicola* respectively, can reside within seeds, facilitating their long-distance spread and acting as a source of infection in various countries. Controlling the phytosanitary quality of seeds is crucial to prevent pathogen dissemination and outbreaks. The International Seed Testing Association (ISTA) developed the 7-007 method to detect *Alternaria linicola*, *Colletotrichum lini*, and *Botrytis cinerea* on flax seeds. This method consists of plating seeds on a medium, incubating the plate for nine days and carrying out morphological identification. This method is only used for the detection of *Alternaria linicola*, *Colletotrichum lini*, and *Botrytis cinerea*. While not officially validated, *Fusarium* spp. and *Boeremia exigua* var. *linicola* can also be identified using this same approach on flax seeds. Inspired by ISTA 7-007, GEVES, the French national reference laboratory, uses a similar method to detect these five key taxa in flax seeds (*Alternaria linicola*, *Colletotrichum lini*, *Botrytis cinerea*, *Fusarium spp*., and *Boeremia exigua* var. *linicola*). The inclusion of these five fungi in the GEVES method offers a more comprehensive approach to pathogen detection compared and respects the following current European Regulation (EU) 2016/2031 compared to the existing ISTA method. In order to characterize the reliability of this method, GEVES evaluated the performance criteria of this method namely, analytical sensitivity, diagnostic sensitivity and specificity, accuracy, repeatability and reproducibility. The successful validation of this method for the causal agents of these five important diseases will open doors to potential improvement of the ISTA method. This advance provided at international level through ISTA will help to limit the spread of these diseases in flax cultivation and promote healthier crops.

Keywords: detection, flax, seed, mycology, method.

Pr03

Session: Development and validation of tests

Development of a multiprong real-time PCR test for the detection of the grapevine pathogen *Xylophilus ampelinus*

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Xylophilus ampelinus, the casual agent of grapevine bacterial blight, reduces yields and poses a barrier to international trade in planting material. Reliable detection is critical, but the pathogen's slow growth and the inconsistent test results for latently infected material hinder effective diagnosis. Using a genome-informed strategy, we identified unique genetic markers and designed several real-time PCR tests targeting *X. ampelinus*. These tests were assessed for specificity, sensitivity, and robustness, and their performance validated across grapevine tissues (leaves, roots, xylem) and in a test performance study. Three tests, Xamp_BA_2, TXmp22.4, and Xamp_BA_7, demonstrated high specificity and sensitivity without cross-reactivity to non-target bacteria. Among them, Xamp_BA_2 provided the most consistent diagnostic performance, achieving > 97% sensitivity across diverse matrices; therefore, this test is recommended for routine diagnostics, with the other tests serving as complementary confirmatory tools. The tests substantially strengthen the diagnostic toolkit for *X. ampelinus*, enabling accurate detection even in woody tissues which are commonly tested for latent infections. Their robustness across laboratories highlights their suitability for phytosanitary programs. By improving reliability of detection, these tools can help limit the spread of grapevine bacterial blight, safeguard trade, and support the long-term sustainability of vineyards.

Keywords: *Xylophilus ampelinus*, grapevine bacterial blight, real-time PCR, latent infection, phytosanitary testing.

Pr04

Session: Development and validation of tests

Development and Validation of a real-time PCR kit for the detection of the *Ralstonia Solanacearum* Species Complex (RSSC)

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The *Ralstonia solanacearum* species complex (RSSC) is a globally significant phytopathogen complex responsible for bacterial wilt in a wide range of economically important crops. Rapid and accurate detection is critical for effective disease management. This presentation outlines the development and validation of a real-time PCR kit specifically designed for the detection of the *Ralstonia solanacearum* species complex. The test targets conserved genomic regions across the species complex, ensuring broad-spectrum detection while maintaining high specificity. Validation was performed using a diverse panel including a number of strains of each of the three species in the RSCC i.e. *R. solanacearum*, *R. pseudosolanacearum,* and *R. syzygii.* and non-target organisms, demonstrating robust sensitivity, reproducibility, and diagnostic specificity. The kit's performance was further evaluated in field samples from various geographic regions, confirming its utility for routine surveillance and phytosanitary applications. This real-time PCR kit provides a reliable tool for early detection of RSSC and supports routine surveillance and phytosanitary diagnostics.

Keywords: Real-time PCR kit, *Ralstonia solanacearum* species complex (RSSC), diagnostic validation, bacterial wilt, phytopathogen detection

Pr05

Session: Development and validation of tests

The Italian Experience with *Pantoea stewartii* subsp. *stewartii*: Genomics, Diagnostics, and Insect-Mediated Transmission

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Pantoea stewartii subsp. *stewartii* is the causal agent of Stewart's wilt of maize (*Zea mays*), a quarantine disease causing severe economic losses. Native to North America, *P. stewartii* subsp. *stewartii* has spread globally via the maize seed trade, and its presence has been reported in Italy since 2015. Reliable diagnostics are essential to distinguish *P. stewartii* subsp. *stewartii* from the non-pathogenic *P. stewartii* subsp. *indologenes*, occasionally associated with maize seeds. Interlaboratory comparisons were carried out to evaluate the sensitivity, specificity, and accuracy of real-time PCR tests for seed testing. Italian isolates collected in 2015 and 2018 were further characterized through biochemical, pathogenicity, and molecular analyses, and their genomes sequenced using MinION and Illumina platforms. Genomic data revealed introgression events and supported the development of a new real-time PCR test. This test demonstrated high analytical sensitivity (10^3 CFU/mL) and specificity, significantly improving the discrimination between *P. stewartii* subsp. *stewartii* and *P. stewartii* subsp. *indologenes*. Besides seed transmission, insect vectors play an important role in the epidemiology of Stewart's wilt. While in North America *P. stewartii* subsp. *stewartii* is primarily spread by the corn flea beetle (*Chaetocnema pulicaria*), this vector is absent in Europe, prompting the search for alternative transmission routes. In Italy, the invasive brown marmorated stink bug (*Halyomorpha halys*) tested PCR-positive for *P. stewartii* subsp. *stewartii*, and preliminary studies have been conducted to assess its potential as a vector. These findings expand genomic knowledge of Italian *P. stewartii* subsp. *stewartii* isolates, enhance diagnostic reliability, and provide new insights into possible insect-mediated transmission, contributing to improved risk assessment and phytosanitary strategies in Europe.

Keywords: PSS, diagnostic method, maize, vector, plant pathogens, genome sequencing.

Pr06

Session: Development and validation of tests

Development and validation of real-time PCR for in-wood detection of *Ceratocystis ficicola*, the agent of canker, wood discolouration and wilt of the common fig tree (*Ficus carica*)

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Ceratocystis ficicola is the agent of a lethal disease of fig trees characterized by bark canker, wood discolouration and wilt, which was noted for the first time in Japan in the 1970's where it is still present and severely damaging fig plantations. Recently, *Ceratocystis ficicola* has been reported in diseased fig trees in two EU countries (Greece and Italy), thus alerting the Mediterranean National Plant Protection Organizations (NPPOs) and the European and Mediterranean Plant Protection Organization (EPPO). The pathogen was included in the EPPO Alert List and successively in the A2, list thereby qualifying as a quarantine pest for which regulation is recommended. A critical point for effective monitoring of the pathogen spread is the difficulty in isolating the fungus from diseased plants and the lack of reliable diagnostic tools. To fill the gap, we developed and validated in-house a real-time PCR test based on the intercalating dyes, EvaGreen and SYBR Green. The primer pair was designed in the internal transcribed spacer (ITS) region. All phases of the work were thoroughly verified according to the basic criteria of repeatability and reproducibility. Amplification efficiency and selectivity were characterized with amplifications of serial dilutions of the fungal genomic DNA either alone or spiked with extracts of three types of fig wood - healthy-looking, necrotic and dried necrotic - and resulted (nearly) best fitting, and reproducible. In fact, efficiency ranged from 95.7 to 102.6%, demonstrating that the amplification process was properly optimized and that the matrix did not negatively affect PCR efficiency. The test was able to detect a gDNA quantity per PCR reaction as low as 10 fg (Limit of Detection) for EvaGreen test and 3 fg for SYBR Green test, in a Ct range of 35-36. Full repeatability and reproducibility were also verified in the presence of extracts from the host matrix. The test was shown to be completely exclusive when tested on *Ceratocystis platani* and on additional 49 non-target fungal taxa isolated from fig wood. It had 100% inclusivity when tested on *Ceratocystis ficicola* isolates from Sicily (Italy) and Japan. Diagnostic specificity and sensitivity were thoroughly validated by applying the test on plant samples from artificially-infected fig trees and from trees naturally infected and not-infected with *Ceratocystis ficicola*. Values obtained were best fitting for both parameters.

Keywords: fig tree, *Ceratocystis ficicola*, quarantine pathogen, diagnosis, real-time PCR, EvaGreen, SYBR Green.

Pr07

Session: Development and validation of tests

Development and validation of MUSTI; a High Throughput Sequencing test in support of fungal identification using rDNA and selected single copy barcodes

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Accurate fungal identification is crucial for plant health diagnostics, yet conventional approaches based on PCR amplification and Sanger sequencing of ribosomal DNA (rDNA) and single copy barcodes are often iterative, labour-intensive and limited by primer specificity or availability. To overcome these challenges, we developed and validated the Mycological Universal Strain Typing & Identification (MUSTI) test; a high throughput sequencing (HTS)-based diagnostic test that integrates Illumina whole genome shotgun sequencing with standardized bioinformatic and reporting procedures. MUSTI extracts DNA from liquid monosporic or hyphal tip cultures and generates a minimum of 2 Gb sequencing output per sample. From these assemblies, the internal transcribed spacer (ITS) region and commonly used single-copy barcodes (i.e. actin, calmodulin, translation elongation factor 1-alpha, RNA polymerase II second largest subunit, and beta-tubulin) are mined and analysed in support of species identification. The test was validated under EPPO PM 7/98 (5) for analytical sensitivity, analytical specificity, repeatability, reproducibility, and robustness, focusing on regulated species within the Dutch NPPO ISO 17025 accreditation scope for Sanger sequencing and extending across diverse Ascomycota and Basidiomycota regulated species. This work demonstrates a transition from ad hoc bioinformatic pipelines towards a fully standardized diagnostic workflow, covering all steps from culturing and DNA extraction to sequencing, analysis, and reporting. We further outline the role of HTS in future plant health mycology diagnostics, aligning with EPPO PM 7/151 considerations for the use of high throughput sequencing in plant health diagnostics.

Keywords: Diagnostics, high throughput sequencing, test definition, culturable fungi, regulatory plant health.

Pr08

Session: Development and validation of tests

Establishing a validated nanopore sequencing workflow for untargeted plant virus detection in diagnostic settings

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High-throughput sequencing (HTS) has transformed plant virus detection, with nanopore sequencing (Oxford Nanopore Technologies, ONT) emerging in recent years as an alternative to the more broadly applied Illumina sequencing. While Illumina HTS has previously been successfully employed to identify plant viruses globally and within NIB we have also adopted nanopore sequencing using MinION (ONT) device. The workflow employs ribosomal RNA-depleted total RNA and a PCR-cDNA barcoding strategy, enabling untargeted detection of plant viruses in composite samples with performance comparable to Illumina sequencing. To ensure robustness and reliability across diagnostic applications, multiple process controls were incorporated throughout both the wet-lab and bioinformatic protocols. These include: (i) an additional well-characterized sample containing a low-titre virus not expected to be present in the analysed samples (alien control); (ii) artificial ERCC (external RNA controls consortium) spike-in control, with a range of target titres, added to each sample; and (iii) a high titre artificial RNA spiked sample (RCS (RNA control sample)). Using these controls, a rigorous post-sequencing bioinformatics analysis procedure was developed to reliably monitor different aspects of the process: low titre viral detection and cross-contamination (alien control), sequencing performance of each sample (ERCC spike-in control) and quantitative estimation of expected barcode cross-talk for each detected virus (RCS control). Additionally, to continuously evaluate the performance of the bioinformatics pipeline, a previously analysed alien control dataset is reanalysed in each run. Validation was conducted in accordance with EPPO guidelines PM 7/98 and PM 7/151, and the protocol was accredited under ISO 17025. The validation results are available in the EPPO database on Diagnostic Expertise, and a description of the test is included in the EPPO PM 7/151 Standard new supporting information as an example facilitating the adoption of this method by other diagnostic laboratories.

Keywords: plant virus, HTS, nanopore, diagnostics, validation.

Session 2: Advances in diagnostic technologies used in the field and in the laboratory

Pr09

Session: Advances in diagnostic technologies used in the field and in the laboratory

Can Scent Detection Dogs be Used for the Detection of *Erwinia amylovora* and *Cryphonectria parasitica*?

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Erwinia amylovora (the causal agent of fire blight in pome trees) and *Cryphonectria parasitica* (the causal agent of chestnut blight in sweet chestnut trees) are economically important diseases, which are difficult to control. The aim of the project was to enable early disease detection to reduce economic losses. This study investigated the ability of dogs to detect the target (bacterial and fungal) plant pathogens. As part of the project, both shelter dogs and already trained detection dogs were trained and evaluated for their suitability to detect the selected pathogens. The Detection Dog Training System (DDTS) was employed during the training phase to condition the dogs to recognise the scent of the pathogens. Their ability to discriminate between target and various distraction odours was assessed using both the DDTS and line-up tests. Additional training was conducted using infected plants. Under both controlled experimental conditions and field settings, the dogs demonstrated high accuracy in detecting the pathogens. They successfully identified cultures of *E. amylovora* and *C. parasitica*, as well as infected plants, with an accuracy ranging from 84% to 100%, regardless of whether the plants showed visible symptoms. The study found no evidence of contamination of the dogs with the plant pathogens. Additionally, the air around infected and non-infected plants was sampled using a modified vacuum cleaner. Analysis of these samples by trained detection dogs enabled differentiation between infected and healthy plants. Air sampling, being a non-invasive technique, allows rapid screening of large tree populations. The results of the study suggest that detection dogs can serve as a valuable addition to existing plant health measures, offering a fast, sensitive, and non-invasive method for early disease detection.

Keywords: scent detection canine, fire blight, chestnut blight, plant pathogens, disease management.

Pr10

Session: Advances in diagnostic technologies used in the field and in the laboratory

Assessing adoption drivers of a VOC sensor technology for pest and pathogen detection: Insights from a survey among European nurseries

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Global trade networks involving the trade of nursery stock remain a major pathway for the introduction of invasive pests and pathogens, as latent or symptomless infections continue to challenge current biosecurity measures. Extending diagnostic capacity beyond visual inspections and laboratory testing is a key objective of the Horizon EU PurPest project, which strives to develop a novel volatile organic compound (VOC) sensor technology. This sensor serves as an 'electronic nose' and is designed for non-invasive and accurate pest and pathogen detection. We conducted a multi-country survey among nurseries in Italy, France, Germany, and Romania, yielding 420 valid completed questionnaires. Our results reveal a strong reliance on visual inspection methods, diverse experiences with invasive species, and a general trust in the existing plant passport certification scheme. Furthermore, while nurseries broadly recognize the importance of early detection, they tend to assign primary responsibility to exporters, border control points, and national plant protection organizations. Although the potential benefits of the VOC sensor are acknowledged by the majority of respondents, there are concerns over initial investment costs, integration into existing workflows, and trust in its diagnostic performance. These findings provide an empirical foundation for understanding the adoption potential of VOC-based diagnostics at the nursery level, an actor not legally obliged to perform phytosanitary inspections, yet directly interested in ensuring the trade of pest-free plant material.

Keywords: plant health, nursery industry, technology adoption, choice experiment, EU biosecurity.

Pr11

Session: Advances in diagnostic technologies used in the field and in the laboratory

Early detection of the pinewood nematode based on volatile organic compounds (VOCs)

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Early detection of plant pests is critical for preventing large-scale outbreaks, yet conventional techniques such as morphological identification and molecular validation are often time-consuming, labour-intensive, and reliant on advanced expertise. For the quarantine pest *Bursaphelenchus xylophilus* (the pinewood nematode), the causal agent of pine wilt disease, a positive identification can range from 3 to 15 days. Emitted volatile organic compounds (VOCs) are now being analyzed as biomarkers for the early-stage detection of plant pests. In the present work, we analyzed VOCs emitted by in vitro monoxenic cultures of the pinewood nematode, feeding on *Botrytis cinerea* fungal mats, to profile nematode specific volatiles. Several compounds were identified through thermal desorption coupled to gas chromatography - mass spectrometry (TD-GC/MS) as linked to pinewood nematode population growth. The main emitted VOCs were 2-methyl-4-heptanone and 2-methyl-4-heptanol, potential aggregation pheromones. Analytical platforms such as TD-GC/MS enable sensitive, non-destructive characterization of volatiles from these types of samples, while emerging sensor-based technologies (e-noses, portable VOC detectors) present opportunities for field-deployable surveillance. The application of volatile profiling not only facilitates early diagnosis but also enhances understanding host - pathogen dynamics, potentially informing integrated management strategies. This preliminary work underscores the potential of VOC-based biomarkers as a rapid, reliable, and scalable tool for monitoring pinewood nematode infection, paving the way toward more sustainable protection of forest ecosystems.

Keywords: *Bursaphelenchus xylophilus*, Gas chromatography, Pine Wilt Disease, Volatiles.

Pr12

Session: Advances in diagnostic technologies used in the field and in the laboratory

MALDI-TOF mass spectrometry to advance the diagnostics of closely related *Fusarium* species

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Phytopathogenic *Fusarium* species are often difficult to distinguish from closely related species, hampering a reliable and quick diagnosis. Matrix assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometry can be used to distinguish species based on their unique protein profiles and provides an efficient potential to complement morphological and molecular diagnoses. A main spectrum profile (MSP) library was prepared containing eight strains of *Fusarium circinatum*, including both mating types. Additionally, 76 strains were included of a total of 32 *Fusarium* species that are morphologically closely related and have the same host range. The MSP library was complemented with high throughput sequence analyses to validate the diagnostic power of the MALDI-TOF mass spectrometry. The results of this work provide evidence that MALDI-TOF mass spectrometry can be applied to identify specific plant pathogenic fungi. Thus far these fungi have been isolated and grown in pure culture before analysis. With adaptations, even obligate fungal pathogens that cannot be cultured, could be analysed by MALDI-TOF mass spectrometry in the future. MSP libraries are highly transferable between laboratories as long as compatible equipment is being used. This makes the MALDI-TOF technique suitable for diagnostic networks whilst experience and libraries can be exchanged and developed in interlaboratory collaborations.

Keywords: *Fusarium circinatum*, MALDI-TOF, mass spectrometry, validation, species distinction.

Pr13

Session: Advances in diagnostic technologies used in the field and in the laboratory

New molecular tools for early detection of forest pests and pathogens

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Plant diseases represent a serious threat to plant productivity, food security, and natural ecosystems. An effective framework for early warning and rapid response is a crucial element to mitigate or prevent the impacts of biological invasions of plant pests and pathogens. For these reasons, molecular detection tools play an important role in monitoring plant health, surveillance, and quantitative pests and pathogens risk assessment, thus improving best practices to mitigate and prevent plant pests and pathogens threats. Considering prevention to be the best strategy to protect plants from diseases and insect outbreaks, this talk focuses on fast and reliable methods to detect and identify the presence of woody plant pests and pathogens at early stage of disease development before symptoms occur in the host. A harmonized pool of novel technical, methodological, and conceptual solutions is needed to prevent entry and establishment of new diseases in a country and mitigate the impact of both invasive and indigenous organisms to forest ecosystem biodiversity and productivity.

Keywords: Invasive species, Real-time PCR, LAMP, eDNA, Quarantine pests.

Pr14

Session: Advances in diagnostic technologies used in the field and in the laboratory

Pushing the boundaries of in-field plant pathogen detection: Applying CRISPR-Cas technology for point-of-need testing for a resistant strain of *Phytophthora infestans*

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DNA based detection methodologies are increasingly being applied across the spectrum of diagnostics including plant pathogen monitoring. Targeting DNA (or RNA) offers a level of specificity that is not always possible with antibodies. While PCR and DNA sequencing-based methods are a robust approach, these are more suited to central laboratory testing due to their associated energy and cost requirements. More recent advances in isothermal techniques have forged a path for developing in-field testing kits. Among these, Loop-Mediated Isothermal Amplification (LAMP) or Recombinase Polymerase Amplification (RPA) have been a popular approach with some limitations. Inspired by advances in clinical diagnostics, we coupled RPA with CRISPR-Cas technology for the detection of various target species with a view to developing on-site testing solutions. RPA-CRISPR-Cas requires a temperature of just 37°C and offers the most superior specificity of all currently available isothermal methods. We have successfully developed an RPA-CRISPR-Cas test that can exclusively detect late potato blight (*Phytophthora infestans*) using either fluorescence or lateral flow detection (akin to Covid19 antigen type test) formats. More recently, we have advanced our testing capability further, through the development of a lateral flow test that can exclusively detect a resistant strain of *Phytophthora infestans*. These DNA based tests can be used on any sample type, including infected potato leaves or a bioaerosol derived environmental DNA sample.

Keywords: Potato, Blight, CRISPR, Resistant, On-Site.

Pr15

Session: Advances in diagnostic technologies used in the field and in the laboratory

Portable advanced molecular technologies for detection of plant pathogens

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The rapid detection of plant pathogens is essential to support surveillance programs, guide disease management strategies, and safeguard plant trade at border control posts. Advanced molecular diagnostic technologies, while accurate and efficient, are often linked to laboratory facilities and specialized non portable equipment. Moreover, early detection and rapid response against emerging threats require new validated tools able to be applied in a real diagnostic on-site context. The Emergent Plant Disease Prevention and Management Team group at IVIA is implementing its vast diagnostic experience in the development and validation of next generation portable tools. These include nanopore sequencing (Oxford Nanopore Technologies), recombinase polymerase amplification (RPA), and magnetically induced PCR platforms, all of which allow rapid, sensitive, and specific detection of fungi, bacteria, and viruses directly from plant material. These tools will combine laboratory diagnostics robustness with on-site speed and versatility, facilitating early detection and rapid response against emerging threats.

Keywords: On site Diagnostics, Plant pathogens, RPA, HTS-ONT, Magnetic induction PCR.

Pr16

Session: Advances in diagnostic technologies used in the field and in the laboratory

Can Nanopore Sequencing Transform Border Biosecurity Diagnostics?

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Onsite diagnostic tools often rely on technologies that are highly targeted and inflexible, limiting their ability to detect a broad range of pests. However, advancements in high throughput sequencing (HTS) and portable platforms such as the MinIon (Oxford Nanopore) offer a potential alternative: real-time, on-site sequencing at points of inspection. In collaboration with animal health specialists working on notifiable diseases of livestock and aquaculture, we are exploring this potential across the 'One Health' spectrum. This presentation will highlight progress in applying nanopore sequencing within the UK Plant Health inspection landscape, with a particular focus on identifying insect eggs of priority pests using a semi-targeted approach. We will share information on method development for targeted nanopore sequencing, including mitochondrial enrichment via rolling circle amplification. Additionally, we will address the practical challenges of deploying advanced genomic technologies at borders and explore the potential costs and benefits of integrating these tools into biosecurity workflows.

Keywords: Biosecurity, high throughout sequencing, onsite diagnostics, rolling circle amplification, pest identification.

Pr17

Session: Advances in diagnostic technologies used in the field and in the laboratory

Exploring Oxford Nanopore Sequencing Technology (ONT) Metabarcoding in Phloem Bacteria Diagnostics: Opportunities and Challenges

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Effective disease management relies on rapid and accurate detection and identification of pathogens. However, this process can be challenging, particularly for phloem-restricted bacteria such as '*Candidatus* Phytoplasma' and '*Candidatus* Liberibacter', which cause significant damage to a wide range of crops. The '*Ca*. Phytoplasma' genus exhibits a high genetic diversity, with numerous 16Sr RFLP (sub)groups, species and strains, while '*Ca*. Liberibacter', though comprising fewer pathogenic species, displays complex genetic lineages or haplotypes. Both groups infect a broad spectrum of plants, causing both similar as well as diverse symptoms. In some cases, they coexist within the same host, making symptom-based diagnosis unreliable. Current diagnostic methods rely on multiple consecutive (nested) PCR tests combined with Sanger sequencing for '*Ca*. Phytoplasma' and '*Ca*. Liberibacter' species identification, RFLP analysis for '*Ca*. Phytoplasma' (sub)group classification and sequence type SNP analysis for '*Ca*. Liberibacter' haplotyping. Altogether, these methods are time-consuming, particularly when both pathogen types are suspected. Oxford Nanopore Sequencing Technology (ONT) offers a promising alternative, enabling multiplexed PCR tests and facilitating simultaneous pathogen detection in mixed-infection samples. This approach has the potential to reduce both time and cost without compromising accuracy. In this study, the potential of ONT-based metabarcoding for detecting and identifying '*Ca*. Phytoplasma' and '*Ca*. Liberibacter' species was evaluated. Barcode regions for '*Ca*. Phytoplasma' (16S, rplV, secA, and tufB) and '*Ca*. Liberibacter' (16S, 16S-23S, and 50S) were evaluated and selected through *in silico* analysis. High-quality databases were established and curated, and primers were assessed, with new primers developed where necessary. PCR tests were optimized by determining the optimal annealing temperatures and primer concentrations. The ONT library preparation and bioinformatics workflow were designed and refined using a range of samples, including mock, uninfected, spiked, single- and mixed-infected and serially diluted samples, with both simplex and multiplex PCR approaches. The results highlighted the potential of ONT metabarcoding but also revealed challenges, particularly the lack of well-curated reference databases and sensitivity issues that compromise the reliability of taxonomic assignments. The study ultimately proposes a flow diagram approach for both '*Ca.* Phytoplasma' and '*Ca.* Liberibacter'. For '*Ca*. Phytoplasma', the approach is tailored to the 16Sr species groups and moves away from fixed thresholds.

Keywords: '*Candidatus* Phytoplasma', Oxford Nanopore Sequencing (ONT), Multi-Locus Sequence Analysis (MLSA), DNA barcoding, Diagnostics.

Session 3: Diagnostics of emerging & re-emerging pests

Pr18

Session: Diagnostics of emerging & re-emerging pests

Molecular diagnostic tests to strengthen seed systems

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One major constraint in yam (*Dioscorea spp*.) production is the limited amount of virus-free planting material available and its high cost. Propagation through 'seed' yam tubers encourages the use of infected planting materials, increasing virus incidence and yield losses, a common issue for vegetatively propagated crops. Yam farmers in West Africa often use low-quality, virus-infected planting material from their own or neighbouring farms. Scientists at the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) in Ghana have utilised an aeroponics and hydroponics system to enhance seed yam production. This system has a high multiplication rate, generating thousands of plantlets from a single plant. Although virus titres in the plantlets are reduced, they are not eliminated, complicating reliable virus detection and making it difficult to ensure that planting material is virus-free. This can lead to false virus-negative certification of planting materials, so that infected materials continue to be spread and threaten crop production and food security. We have developed and evaluated isothermal amplification- and high throughput sequencing (HTS)-based diagnostic tests for virus detection in various crops. Over 40 viruses are known to infect yam, with many of the new viruses identified using HTS technologies. The diagnostic tests for yam developed at Natural Resources Institute (NRI) have been transferred to CSIR-CRI, improving their capacity in diagnostics and seed certification and contributing towards the production and sustainable supply of high-quality seed yams. The details and potential wider application of these tests is discussed as well as the factors influencing their future deployment in support of seed systems of root and tuber crops.

Keywords: LAMP diagnostics, High-throughput sequencing (HTS), MinION sequencing, Vegetatively propagated crops.

Pr19

Session: Diagnostics of emerging & re-emerging pests

Surveillance, symptomology, and status update of three emerging viruses in Trentino region in Northern Italy

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Trentino, in Northern Italy, is a major European hub for the production of pome fruits and grapevine, as well as stone fruits. Maintaining high phytosanitary standards is essential to sustain both economic viability and export potential. This study reports on the current status of three globally emerging viruses detected in Trentino: *Luteovirus mali* (*Apple luteovirus* 1, ALV1), *Robigovirus necroavii* (Cherry necrotic rusty mottle virus, CNRMV), and *Trichovirus pinovitis* (Grapevine Pinot gris virus, GPGV). Field surveys were combined with molecular diagnostics to assess their incidence and distribution. This updated overview contributes to regional risk assessment and underlines the importance of integrating surveillance with advanced diagnostics for the management of emerging viral threats in fruit production systems.

Keywords: emerging virus, *Apple luteovirus*, Cherry necrotic rusty mottle virus, Grapevine Pinot gris virus.

Pr20

Session: Diagnostics of emerging & re-emerging pests

Strengthening Avocado Disease Surveillance through Enhanced Diagnostics for Scab and Laurel Wilt

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Avocado production is increasingly threatened by two major diseases: avocado scab, caused by *Elsinoe perseae*, and laurel wilt, caused by *Harringtonia lauricola*. Both pose significant biosecurity risks with the potential for severe economic and ecological impacts. While scab has historically been considered a minor problem in certain cultivars, its significance is rising as climate change and expanded cultivation into humid regions create more favourable conditions for disease development. In contrast, laurel wilt has already devastated thousands of acres of avocado in Florida and continues to spread through natural and human-mediated pathways, endangering avocado industries throughout the Americas. This presentation introduces novel diagnostic tools that enhance early detection and surveillance of these emerging threats. The new tests enable direct pathogen identification from infected tissues, greatly reducing diagnostic turnaround time compared to traditional culturing and morphological methods. For avocado scab, molecular tests now distinguish true infections from mechanical or insect damage, improving accuracy in field assessments. For laurel wilt, a field-deployable molecular protocol was developed to detect *H. lauricola* directly from symptomatic trees and vector beetles, providing a valuable tool for orchard monitoring and biosecurity planning. By focusing on diagnostic innovation, this work underscores the critical role of rapid and precise pathogen detection in effective disease management. These advancements not only empower growers to make timely management decisions but also strengthen regional and international surveillance networks. Integrating these diagnostic tools into monitoring programs will bolster avocado disease management, safeguard production systems, and promote sustainable agricultural practices amid growing phytosanitary challenges.

Keywords: Pathogen detection; Rapid diagnosis; Biosecurity, Field-diagnosis.

Pr21

Session: Diagnostics of emerging & re-emerging pests

Development and validation of PCR tests to diagnose the new *Trichoderma* ear rot on maize

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Maize (*Zea mays*) is one of the most extensively cultivated crops on the planet. Nevertheless, the maize plant is prone to a variety of fungal diseases, including ear rots. Recently, a new Trichoderma ear rot disease has been described on maize in southern Germany, with mounting evidence indicating that this emerging disease is triggered by high temperatures during the dry summer months. The symptoms manifest predominantly from the stage of fructification onwards, initially as white mycelium on the maize cobs, which turns green to grey-green through sporulation, spreading between or covering the kernels. Stalks are also susceptible to infection, albeit with reduced frequency. The impact of this disease on maize production can be significant, potentially resulting in important yield losses, a notable decrease in cob weight, and impaired seed germination. Subsequent reports of the disease have been documented in several European (France, Italy, Malta, Austria) and Asian (China, India) countries. In all of these cases, *Trichoderma afroharzianum* was identified as the causal agent. Consequently, the accurate identification of *T. afroharzianum* is imperative for the effective monitoring and management of this novel Trichoderma ear rot. The objective of this study is to provide a rapid and sensitive tool for this purpose. Molecular tests using conventional and real-time (TaqMan) PCR have been developed based on the TEF and RPB2 regions respectively. The validation procedure was conducted in accordance with EPPO Standard PM 7/98. Results obtained demonstrated the reliability of both tests in detecting *T. afroharzianum* from pure cultures and infected maize kernels, and their capability for early detection. This diagnostic tool is essential for developing targeted management strategies to address this emerging threat to maize production.

Keywords: *Trichoderma afroharzianum*, corn ear rot, emerging disease, Taqman PCR.

Pr22

Session: Diagnostics of emerging & re-emerging pests

First report of *Verticillium dahliae* causing wilt of Pawpaw (*Asimina triloba*)

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Verticillium wilt, caused by the fungal pathogen *Verticillium dahliae*, is a significant disease affecting a wide range of vegetable, field, ornamental, and tree crops. Pawpaw (*Asimina triloba*) is a recently introduced fruit crop in Austria. In 2022, symptoms of wilting were observed in a four-year-old commercial plantation located in Lower Austria. Affected trees exhibited leaf yellowing, wilting, blight of leaves and branches, and browning of vascular tissues. The pathogen *V. dahliae* was isolated from symptomatic branches and identified through DNA barcoding. Additionally, direct detection from plant tissue DNA was performed using species-specific real-time PCR. Soil analysis revealed a high density of *V. dahliae* microsclerotia in the affected site. To confirm pathogenicity according to Koch's postulate, inoculation of healthy *Asimina triloba* plants and subsequent re-isolation of the pathogen were performed. To the best of our knowledge, this is the first report demonstrating the ability of *V. dahliae* to colonize *Asimina triloba* and to induce disease symptoms under field conditions.

Keywords: *Verticillum dahliae*, Verticillium wilt, *Asimina triloba*, Pawpaw, first report.

Pr23

Session: Diagnostics of emerging & re-emerging pests

Bacterial Disease Outbreaks in Protected Crops and Nurseries: An Emerging Challenge

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In recent years, bacterial diseases have emerged as a significant and increasing threat in nursery production and protected cultivation systems in Italy. Factors such as climate change, intensified production practices, global plant trade, and the use of high-density propagation systems have contributed to the spread and establishment of bacterial pathogens in these controlled environments. Pathogens such as *Pseudomonas* spp. and *Xanthomonas* spp. have been increasingly reported in a wide range of ornamental and horticultural crops particularly in regions with high horticultural intensity, including Northern Italy and other Mediterranean countries. These pathogens not only cause direct yield and quality losses but also pose significant challenges for certification systems and phytosanitary regulations. The purpose of this work is to explore the recent emergence and dynamics of bacterial diseases in nurseries and greenhouse crops, with a focus on the Italian experience. Special attention is given to the contributing factors, the diagnostic and regulatory gaps, and the need for integrated strategies tailored to high-value production systems in Italy.

Keywords: Plant bacterial pathogens, Greenhouse systems, Disease emergence, *Pseudomonas* spp., *Xanthomonas* spp.

Session 4: Plant Health diagnostics from a broader perspective

Pr24

Session: Plant Health diagnostics from a broader perspective

Development of diagnostics for inspectors

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For decades, many new technologies have been shown to be field deployable for the detection and in some cases identification of plant pests. Despite this, there are few examples where technology has been developed into field tests, and even fewer examples of tests which are routinely used by inspection services. This presentation will provide an inspector's view on possible opportunities to generate new and potentially better samples for diagnosticians and tests for inspectors; Why from an inspector's view it is not always possible to get the best sample for testing, including why sampling strategies are not always possible; How inspectors spot and take good samples, and why they may not always be the best; How diagnostic results are used outside the laboratory and the consequences of taking a sample and whether sampling is always a good thing and what good diagnostic reporting looks like for inspectors. Finally, the presentation will suggest four discussion steps which have proven to be useful in taking new technology from concept to field deployment. It will also describe factors for consideration which have been identified as barriers to deployment and give examples where deployment has been successful.

Keywords: Detection, inspection, new technologies.

Pr25

Session: Plant Health diagnostics from a broader perspective

Applying different workflows in plant virus diagnostics

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Plant health diagnostic laboratories such as NVWA-NIVIP are responsible for processing a wide variety of samples from annual surveys, import/export inspections or plant clinics, covering a broad range of (regulated) pest and host combinations. To obtain a reliable virus diagnosis covering these combinations, laboratories implement diagnostic workflows containing detection and identification steps. In plant virology, workflows often consist of one or more diagnostic test(s) because a single test is often not able to provide sufficient assurance for diagnosis. To determine if a test is suitable for its intended use a validation should be performed in accordance with EPPO Standard PM 7/98. Depending on the diagnostic question workflows may either follow a targeted approach with selected tests or a non-targeted approach for which expert knowledge and experience is crucial. For each sample a hypothesis is formed based on sample information such as host, symptomatology, origin and further supported by literature and images. Finally, by combining all results and taking into account contextual information solid conclusions are drawn. We will present case studies illustrating how workflows are applied to obtain a virus diagnosis within plant virology.

Keywords: diagnostic workflow, detection, identification, targeted, non-targeted.

Pr26

Session: Plant Health diagnostics from a broader perspective

From detection to risk assessment: Understanding the fate of plant viruses in organic waste fertilizers

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The increasing use of organic waste fertilizers, such as compost, ‘compost tea’, and insect frass, in sustainable agriculture brings new opportunities for improving soil health and recycling nutrients. However, it also raises important questions about plant health and safety, as persistent plant viruses and viroids may remain infective in these fertilizers and pose a risk of transmission to crops. Reliable detection and risk assessment of these pathogens is therefore essential for safe use of organic fertilizers. In our research, we are investigating whether, and under which conditions, plant viruses and viroids can persist in various organic waste-derived fertilizers, and how this affects their potential transmission to plants. To address these questions, we are developing and testing a combination of molecular diagnostic methods (such as real-time PCR and high-throughput sequencing) and biological assays to detect and assess the infectivity of these pathogens. Our approach includes experiments in controlled laboratory and pilot conditions, as well as studies in plant growth substrates where we monitor whether viruses and viroids from plant residues remain infective in the substrate after removal of infected plants and assess their potential transmission to new plants over time. We will present the concept of our research and the results obtained so far, which will offer new insights into the persistence and infectivity of plant viruses in organic waste fertilizers and will support the development of reliable diagnostic protocols and risk assessment strategies for safer and more sustainable plant health management.

Keywords: fertilizers, plant viruses, viroids, detection, survival.

Pr27

Session: Plant Health diagnostics from a broader perspective

Challenges for diagnosis of tropical root-knot nematodes with a focus on *Meloidogyne enterolobii*

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The tropical root-knot nematode *Meloidogyne enterolobii* was added to the EU quarantine pest list in April 2022 due to its ability to infest many important agricultural crops, including pepper, tomato, cucumber, potato, as well as various woody and herbaceous plants. Furthermore, *M. enterolobii* can reproduce on tomato and pepper cultivars that are resistant to other root-knot nematode species, raising the risk of severe crop damage. *M. enterolobii* poses an emerging global threat, with presence reported in several (sub)tropical regions across North, Central, and South America, Africa, and Asia. In 2023, *M. enterolobii* was detected during an export certification inspection on *Ficus microcarpa* plants in the Netherlands. The affected plants showed root galls, and all plants in the greenhouse from the same consignment were destroyed. These infested plants were imported from China. Following this finding, a survey was conducted among all Dutch growers of *Ficus microcarpa*, revealing *M. enterolobii* in three out of 33 inspected companies. Accurate diagnosis of *M. enterolobii* is challenging due to its high intraspecific variability and morphological similarity to other tropical root-knot nematode species often found in the same sample. Therefore, reliable and sensitive molecular identification techniques are essential. In 2023, discrepancies between morphological and molecular diagnostic results were observed at NVWA-NIVIP. This prompted a comparison of the standard real-time PCR test used by NVWA-NIVIP with an alternative real-time PCR test from the literature, followed by validation of the new test. The results, experiences, and future directions are discussed.

Keywords: *Meloidogyne enterolobii*, real-time PCR, morphology, validation, survey.

Pr28

Session: Plant Health diagnostics from a broader perspective

Development of an early detection protocol for *Erwinia amylovora* from corbicular pollen to monitor its spread in apple orchards

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Fire blight, a severe disease caused by *Erwinia amylovora*, poses a substantial threat to apple production worldwide. As honeybees are key vectors of *E. amylovora*, a real-time PCR-based method was developed for the early detection of the bacterium in corbicular pollen (the pollen baskets on the legs of bees). Sterile pollen was artificially contaminated using a suspension of *E. amylovora* Ea21, a rifampicin-resistant strain, at concentrations ranging from $1x10^8$ to 1 CFU/mL. Non-contaminated pollen served as the negative control. One gram of each inoculated sample underwent serial dilution and plating on Nutrient Agar supplemented with rifampicin to determine CFU counts. Simultaneously, 30 g of inoculated pollen was homogenised in 120 mL of 0.85% (w/v) NaCl with 0.001% (v/v) TWEEN®80 and kept on ice under orbital shaking (140 rpm). After one hour, samples were centrifuged to remove debris, and the supernatants were recovered and further centrifuged. Resulting pellets were resuspended in Tris-HCl buffer (pH 8) and subjected to thermal lysis. DNA was extracted using the DNeasy® mericon® Food Kit (QIAGEN) and analysed via real-time PCR, following EPPO Standard PM 7/20. To assess robustness, the method was applied to more than 200 corbicular pollen samples collected in 2022 and 2023 from Valsugana and Val di Non, two apple-growing locations in Trentino. Overall, the assay enabled reliable quantification of *E. amylovora* in corbicular pollen down to 10^2 CFU g^{-1}. This protocol represents a promising early warning tool for the presence of *E. amylovora*, potentially enabling proactive fire blight management in apple orchards.

Keywords: *Erwinia amylovora*, corbicular pollen, honeybee-mediated dispersal, *Malus domestica*, early warning.

Pr29

Session: Plant Health diagnostics from a broader perspective

Revealing diagnostic gaps and sequence diversity in *Xanthomonas* pathovars of common bean through molecular testing and DNA barcoding

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We systematically evaluated diagnostic tests for two common bean pathogens, *Xanthomonas phaseoli* pv. *phaseoli* (XANTPH) and *X. citri* pv. *fuscans* (XANTFF). The evaluation included MALDI-TOF, conventional PCR, three real-time PCR tests, and DNA barcoding using a panel of 34 bacterial isolates. All PCR-based tests showed 100% exclusivity with no cross-reactions, while MALDI-TOF produced two false positives but proved, useful as a preliminary screening tool by narrowing down candidates for further testing. None of the PCR-based tests detected genetically distinct *X. phaseoli* pv. *phaseoli* isolates from lablab bean. Furthermore, Audy's conventional PCR and the He-ff real-time PCR failed to detect certain *X. citri* pv. *fuscans* isolates; conventional PCR missed the type/pathotype strain, while He-ff failed to detect a highly pathogenic isolate from La Réunion. These diagnostic gaps raise concerns for both routine testing and surveillance, underscoring the need to optimize current tests for improved detection of geographically and genetically divergent isolates. DNA barcoding was the most effective method, with both *gyrB* and *avrBs2* markers independently enabling accurate identification of all *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans* isolates. However, a dual-database approach (EPPO-Q-bank and NCBI GenBank) was required, and reliance on similarity-based matching exposed limitations due to incomplete or inconsistent reference data. To overcome these constraints, we applied diagnostic nucleotide characters (DNCs), 16 in *gyrB*, 19 in *avrBs2*, which provided a more robust framework for identification and improved diagnostic resolution by classifying isolates into ten distinct sequevars of *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. This study offers comprehensive insights into the performance and limitations of current diagnostic tools for *X. phaseoli* pv. *phaseoli* and *X. citri* pv. *fuscans*. The integration of DNA barcoding with DNCs enables reliable differentiation, sequevar classification, and resolution where similarity-based identification proves insufficient, delivering practical value for diagnostics and surveillance.

Keywords: *Xanthomonas phaseoli* pv. *phaseoli*, *Xanthomonas citri* pv. *fuscans*, common bacterial blight; DNA barcoding, diagnostic nucleotide characters.

Pr30

Session: Plant Health diagnostics from a broader perspective

Detection of *Xylella fastidiosa* in spiked dormant plant material at different growth stages, and in naturally infected olive trees with canopy vigour recovery

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In Europe, the spread of various subspecies of the bacterium *Xylella fastidiosa* has become a growing concern, impacting not only olive cultivation but also other plant species of significant economic and environmental value. Following the first detection of *Xylella fastidiosa* subsp. *pauca* on olive, *X. fastidiosa* subsp. *multiplex* was found on almond, while both subsp. *multiplex* and subsp. *fastidiosa* were detected on grapevine in different areas. Accurate and rapid subspecies detection/identification, reliable across various plant hosts and different seasonal conditions, is crucial for an effective application of phytosanitary measures by NPPOs. To address this, the analytical sensitivity of four molecular diagnostic methods -real-time PCR-Harper, digital droplet PCR (ddPCR-Dupas), tetraplex real-time PCR-Dupas, and real-time PCR-Hodgetts was evaluated for the detection and subspecies identification of *Xylella fastidiosa* subsp. *fastidiosa* in grapevine and *X. fastidiosa* subsp. *multiplex* in almond samples, collected at four different seasonal time points and artificially spiked. ddPCR was confirmed as the most robust and sensitive diagnostic tool capable of detecting up to 10^2 CFU/g of plant tissue for the matrices and sampling periods analysed, making it particularly suitable for the surveillance of plant hosts with low bacterial loads. Moreover, since a recovery of canopy vigour has been observed in olive trees in the Salento region of Italy, we evaluated the dynamics of *X. fastidiosa* subsp. *pauca* across different seasons during the period 2022-2025 in olive trees naturally exposed to high inoculum pressure, showing either signs of canopy vigour recovery or not. No statistically significant variations were observed in the bacterial load of the plants analysed across the different sampling periods. These findings underscore the need for further investigation of canopy vigour recovery, its persistence over time, and its potential relationship with other factors.

Keywords: *Xylella fastidiosa*, diagnostic tests, seasonal bacterial load, analytical sensitivity, canopy vigour recovery.

Pr31

Session: Plant Health diagnostics from a broader perspective

From spiking to routine use: versatile applications of reference materials for *Xylella fastidiosa* diagnostics

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Reference materials are indispensable for reliable plant pest diagnostics, but their utility extends far beyond serving as positive or negative controls. Since 2021, we have developed and applied reference materials for *Xylella fastidiosa*, prepared in accordance with EPPO Standard PM 7/147 (Guidelines for the production of biological reference material) to ensure traceability and comparability. These materials support multiple functions. In-house, they enable validation of tests across diverse host plants, revealing diagnostic gaps such as variable DNA recovery depending on matrix and extraction method. Incorporated into routine workflows, they allow process monitoring: run charts of control performance, help distinguish natural variation from systematic shifts, strengthening internal quality assurance. Within ISO/IEC 17025 flexible scopes, the same materials provide evidence for extending accredited testing to new hosts by documenting recovery efficiencies. Finally, when used in interlaboratory studies, they provide a stable benchmark, reducing variability and enabling meaningful comparison of results between laboratories. By uniting these applications, reference materials become dynamic tools rather than static controls. They underpin method development, routine diagnostics, and accreditation, while also supporting harmonisation across laboratories. Embedding reference materials into ongoing validation, process monitoring and proficiency testing will support the generation of Findable, Accessible, Interoperable and Reusable (FAIR)-compliant data and strengthen diagnostics for *X. fastidiosa* and other regulated pathogens.

Keywords: Reference materials, *Xylella fastidiosa*, Diagnostic validation, Quality assurance (ISO/IEC 17025), EPPO PM 7/147.

Pr32

Session: Plant Health diagnostics from a broader perspective

The National Collection of Plant Pathogenic Bacteria: The use of bacterial culture collections for the provision of reference standards: the cornerstone to accurate bacterial diagnosis

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Accurate diagnoses are dependent on using authentic reference material. The aim of bacterial culture collections is to preserve the original properties of an organism. Also, curators of culture collection are responsible for ensuring that all the organisms they house are correctly identified and appropriately named. Strains obtained directly from culture collections reduce the risk of the wrong, or non-authentic, materials being used by scientists as references for diagnosis and research. Guidelines and standards exist to ensure collections are managed appropriately by curators and help collections' staff provide reference material suitable for its intended purpose. 76 NCPPB strains are referenced in 21 EPPO Diagnostic Standards, and a brief history of the collection and its preservation methods are provided, along with an example of how NCPPB strains are used to produce reference material for use at Fera.

Keywords: NCPPB, Reference Material, collections.

Pr33

Session: Plant Health diagnostics from a broader perspective

Validation of Nucleic Acid extraction internal control procedures for diagnosis

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Plant health laboratories routinely implement a high diversity of nucleic acid extraction methods, driven by factors such as the organism, test purpose, and matrix, which all have their specific characteristics, e.g. inhibitor presence. However, this diversity in factors and the lack of standardised procedures in plant health results in varied methodologies, including in-house and published approaches. EPPO recognised the need to evaluate the nucleic acid extraction process as an important step in routine diagnostics and this resulted in a project (acronym IVENAD), engaging over 30 laboratories, which seeks to inventory and validate quality control procedures for nucleic acid extraction in plant pest diagnosis. The aim is to establish guidelines for (internal) control procedures of the different extraction methods. A desktop study led to the inventory of a 110 articles and 170 protocols provided by 19 laboratories. Despite the absence of a uniform methodology, further prioritisation and categorisation of all the internal extraction control methods that the laboratories are implementing, led to a selection of RNA and DNA extraction methods on 4 different matrices (3 plant and 1 insect) to undergo evaluation against a variety of 21 internal controls, specifically targeting a variety of matrix housekeeping genes. This selection underwent a series of extraction tests in view of a further selection of matrix-target and control combinations that were included in an inter-laboratory comparison (ILC) organised among the project partners. The outcomes of this ILC will be discussed and based on these results, the project aims to deliver a set of (partially) validated nucleic acid extraction procedures, both for RNA and DNA applications (e.g. virus and bacteria detection) in (complex) matrices. In addition, the project results are now being summarized in general guidelines for the use of internal controls in nucleic acid extraction procedures for diagnostics, primarily benefiting plant health diagnostic laboratories, but also National Plant Protection Organisations. The implementation of reliable routine nucleic acid extraction procedures as part of the diagnostic procedures enhances rapid and reliable pathogen/pest detection, enabling timely intervention and preventing potential spread.

Keywords: Plant health diagnostics, Internal amplification controls, monitoring nucleic acid extraction.

Pr34

Session: Plant Health diagnostics from a broader perspective

Interplay of morphological and molecular methods in entomology

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The interplay of morphological and molecular methods in entomological diagnostics presents both opportunities and challenges, particularly in the context of maintaining high-quality reference specimens while enabling reliable DNA-based identification. The European Union Reference Laboratory (EURL) for insects and mites conducted a series of experiments, aimed at optimizing sample handling and storage protocols to support both approaches. Key investigations included the evaluation of common non-destructive DNA extraction procedures and a spike and recovery experiment assessing the contamination potential of storage ethanol in the context of non-destructive DNA extraction and pest-detection with highly sensitive tests. Additionally, a long-term storage experiment explored the effects of various media and temperature conditions on Lepidoptera larvae preservation and subsequent DNA yield. The results provide practical insights into how laboratories can preserve morphological integrity while ensuring high-quality DNA for sequencing and pest-identification via real-time PCR. Recommendations will be offered for specimen handling and storage that minimize contamination risks and maximize the utility of samples for both morphological and molecular analyses - ultimately supporting robust, integrative diagnostics in entomology.

Keywords: Pest detection, reference specimens, non-destructive DNA extraction, contamination, specimen storage and preservation.

Pr35

Session: Plant Health diagnostics from a broader perspective

Over 10 years' experience in potato virus proficiency tests as external proof of diagnostic laboratory competence

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Accreditation of diagnostic laboratories to ISO 17025 (General requirements for the competence of testing and calibration laboratories) means that they must demonstrate their competence in detecting pathogens by regularly participating in proficiency tests (PT). The obligation for laboratories to participate and the lack of providers led to the development of a PT organized by the laboratories own initiative for the six viruses relevant for seed potato certification: *Potyvirus yituberosi* (PVY), *Polerovirus PLRV* (PLRV), *Carlavirus misolani* (PVM), *Potyvirus atuberosi* (PVA), Potexvirus ecspotati (PVX) and *Carlavirus sigmasolani* (PVS). The PT took place for the first time in 2010 between three laboratories in Germany. This has since evolved into a major international proficiency test, with a total of 55 sample sets sent to 37 participating laboratories in 16 countries worldwide in 2024. This presentation summarizes the development of this PT and discusses the results obtained. Each laboratory participates with its own established protocols, ranging from serological methods (ELISA), arrays and rapid tests to real-time RT-PCR. This not only provides important information about the competence of the participating laboratories but also allows comparisons within and between methods. Over the years, many laboratories have switched from the time-consuming cultivation of eye cuttings followed by ELISA to time-saving real-time RT-PCR on potato tubers. PT has helped to identify shortcomings in published methods for the detection of individual viruses, leading to improved detection reliability.

Keywords: ELISA, real-time RT-PCR, PT.

Session 5: Initiatives for improving plant health cooperation in diagnostics

Pr36

Session: Initiatives for improving plant health cooperation in diagnostics

The Italian phytosanitary laboratories network to improve and harmonize diagnostics in plant health

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The incidence and impact of transboundary pests are exacerbating by climate change and globalization, leading to high levels of emergence or re-emergence of new threats. Once pests are established, their eradication is often difficult and requires a significant effort to control. Possibilities to prevent damage require timely collection of information on pest occurrence, spread or outbreaks. In this light, great importance is given to diagnostics, mainly in relation to the need of early and reliable detection of the harmful organisms to prevent their introduction into the territory and spread. Italy is divided into 20 regions, each with a regional phytosanitary service with its own and/or designated laboratories, making coordination activities particularly important, especially in the field of diagnostics. Moreover, the geophysical diversity leads to a wide heterogeneity of key cultures and crops, for which phytosanitary surveillance is needed. To face these specific issues, and in compliance with the new phytosanitary regime defined by Regulations (EU) 2016/2031 and 2017/625 (OCR), a national network of phytosanitary laboratories was officially established in 2021 with a Legislative Decree n.19 on 2021/02/02, implemented by Ministerial Decree n.169819 on 2022/04/13. The present contribution illustrates the organization of the national network of phytosanitary laboratories in Italy and its connection with the European network. Furthermore, the main actions implemented by the network to harmonise and improve diagnostics in plant health are illustrated. These include: (i) organization of proficiency tests at national level for selected organisms of EU relevance; (ii) validation of diagnostic tests made official through technical documents approved and issued by the National Phytosanitary Service; (iii) support for laboratory accreditation according to standard EN ISO/IEC 17025; (iv) the organization of training courses on the use of new or innovative detection methods for specific harmful organisms.

Keywords: phytosanitary, Italy, harmful organisms, diagnostics, accreditation.

Pr37

Session: Initiatives for improving plant health cooperation in diagnostics

European reference laboratories, a strong EU network supporting the plant health sector

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The Official Controls Regulation (OCR) EU/2017/625 lays down the framework for the establishment of European Union Reference Laboratories (EURLs) where there is a recognised need to promote uniform practices in relation to the development or use of diagnostic methods and tests used by EU laboratories for official controls and other official activities. In 2019, to achieve this improvement in the plant health sector, one EURL was established for each of the specific categories of plant pests: bacteria, fungi and oomycetes, insects and mites, nematodes, and viruses, viroids and phytoplasmas. The primary tasks of EURLs include the optimization and validation of diagnostic protocols, which are disseminated through a robust network of European National Reference Laboratories (NRLs). Various activities are organized for the NRLs, such as proficiency testing, training sessions, annual workshops, and the exchange of reference materials. Furthermore, the EURLs actively collaborate with international organizations and institutions, such as EPPO, to share protocols and validation data and contribute to the development or improvement of Diagnostic Standards. Collaborative efforts with countries outside the EU have also helped resolve diagnostic issues through training and tailored advisory services.

Keywords: EU Official Controls Regulation, EURL, network, diagnostic, dissemination.

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Session: Initiatives for improving plant health cooperation in diagnostics

EURL Nematodes: Collaboration to Strengthen and Harmonize Diagnostics

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Core activities of the European Union Reference Laboratory (EURL) for Plant Parasitic Nematodes are the development, validation, and standardization of high-quality methods, ensuring reproducible detection and identification of EU quarantine and emerging nematodes. Examples of these include methods for detection and identification of the EU quarantine nematodes: *Meloidogyne enterolobii*, *M. chitwoodi, M. fallax* and *Nacobbus aberrans*, and *M. graminicola* which is on the EU emergency measures list. However, these methods would be of limited value if they could not be practically implemented across the different reference laboratories. To this end, in addition to working on diagnostic methods, the EURL has produced and published full protocols from extraction up to identification. These are publicly available and therefore directly useable by official and national reference laboratories. Implementation of these comprehensive protocols not only strengthens diagnostic preparedness but also enhances the overall harmonization of testing strategies across Europe, supporting effective surveillance and outbreak responses. It also gives the EU competent authorities and the European Commission the assurance of robust and comparable results within the EU. Furthermore, they reach not only the European laboratory network, but also laboratories at an international level, for example in 2025 alone, these protocols were downloaded more than 6 000 times from the EURL website.

Keywords: Plant-parasitic nematodes, EU quarantine nematodes, EURL diagnostic protocols, testing, surveillance.

Pr39

Session: Initiatives for improving plant health cooperation in diagnostics

How the EURL Bacteriology supports international phytosanitary policy-makers on issues related to plant quarantine bacteria diagnostics

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The European Union reference laboratory for pests on plants on bacteria (EURL-Bacteriology) is a consortium of four laboratories: ILVO (Belgium), CREA-DC (Italy), NIB (Slovenia), and NVWA-NIVIP (The Netherlands). The primary objective of the EURL-Bacteriology is to achieve an overall high level of diagnostic quality at National Reference Laboratories (NRLs) regarding designated priority pests and European Union quarantine pests. The EURL-Bacteriology uses its expertise to advise individual NRLs, EPPO, and the European Commission on issues related to plant quarantine bacteria diagnostics. Notable contributions to the European Commission include assistance in drafting diagnostic annexes for regulations on *Xylella fastidiosa* (EU 2020/1201), *Ralstonia solanacearum* (EU 2022/1193), and *Clavibacter sepedonicus* (EU 2022/1194). Moreover, the EURL-Bacteriology published a diagnostic protocol for detecting *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds to anticipate on changing regulations and to facilitate import testing. The EURL-Bacteriology collaborates closely with EPPO to avoid duplication of work and to disseminate results from its activities. This includes submitting test performance study results and other validation data to the EPPO database on Diagnostic Expertise, as well as providing input to improve EPPO Diagnostic Standards. This strengthens the synergy between EPPO and the EURL-Bacteriology to better serve the NPPOs across the EPPO and the EU region. Specific examples will be highlighted during the conference.

Keywords: EURL, diagnostic protocols, validation, test performance study, quarantine pests.

Pr40

Session: Initiatives for improving plant health cooperation in diagnostics

Bridging Expertise: The Power of Cross-Organisational Collaboration

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Cross-organisational collaboration has become a key driver of innovation, enhanced operational efficiency, and overall organizational success. A notable example is a productive partnership between the European Food Safety Authority (EFSA), the University of Padua (DAFNAE Department), and the European Reference Laboratory for Plant Pests for Insects and Mites (EURL-IM). The EURL-IM supported EFSA's group pest categorisation of non-coniferous Scolytinae by systematically gathering and evaluating all available data on identification of the species proposed by EFSA for regulatory consideration. Accurate diagnostics are essential for the effective implementation of plant health regulations. Additionally, the EURL-IM is enriching the global Scolytinae information database (Scoly-HUB), which was developed by DAFNAE. This includes integrating genetic sequences and morphological identification tools for selected species. Furthermore, the EURL-IM is developing KiBAB, an online interactive key based on the biogeographical data of this database and dedicated to the identification of the genera of Scolytinae considered as invasive in Europe. These activities help bridge the pest risk assessment with the practical implementation of diagnostics tools across the EU National Reference Laboratories (EU-NRLs). This initiative not only reinforces the scientific foundation for the identification of Scolytinae but also expands the collaborative framework to include academic institutions, strengthening ties between regulatory bodies and the research community, and filling many important knowledge gaps.

Keywords: bark beetles, data platform, transnational network, diagnostics, plant health.

Pr41

Session: Initiatives for improving plant health cooperation in diagnostics

Towards a more reliable detection of quarantine *Elsinoe* species on citrus: Beyond sequences, primers and probes

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Elsinoe is a genus with a wide host range across numerous plant families and a global distribution. Species in *Elsinoe* cause scab diseases that can infect leaves and stems, while they also heavily impact the appearance of the fruits, which in turn influences their value in the market. Additionally, *Elsinoe* species may pose regulatory barriers to the export of high-value commodities such as citrus. European countries import a considerable amount of citrus to meet internal demands; however, these imports also increase the risk of introduction of the EU-regulated Elsinoe species, namely *Elsinoe australis*, *E. citricola* and *E. fawcettii*. Difficulties in isolating the pathogen and existing knowledge gaps in their biology, species delimitation, host specificity, and geographic distribution are the main factors limiting accurate and timely diagnosis. In response to these challenges and the necessity to develop species-specific detection tests, the EURL Fungi expanded its collaboration network by participating in multidisciplinary international initiatives that are actively investigating the diversity, ecology, taxonomy and detection of *Elsinoe* species. Participation in these networks enabled access to additional genomic data, expertise and biological material, all of which are crucial for developing reliable and robust detection tests targeting the EU-regulated *Elsinoe* species. We will discuss how these multidisciplinary initiatives enhance the capacities of the EURL Fungi and the network, as well as how this framework can be applied to other regulated pathogens within the mandate of the EURLs.

Keywords: CORES group, collaboration, quarantine, scab diseases.

Pr42

Session: Initiatives for improving plant health cooperation in diagnostics

EURL Virology – advancing reliable diagnostics by bringing laboratories together

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To demonstrate the reliability of diagnostic testing, laboratories are generally required to comply with the ISO/IEC 17025 accreditation Standard, which includes proving technical competence and ensuring the validity of results. One of the key methods to demonstrate proficiency is through interlaboratory comparisons. However, formal proficiency testing schemes are often limited or unavailable. In such cases, laboratories must adopt alternative approaches to validate their results. To support this need, the EURL Virology plays a facilitating role by connecting laboratories across and beyond the EU, based on shared methodological or organism-specific needs. These connections enable laboratories to initiate and participate in self-organized interlaboratory comparisons tailored to their specific objectives. A recent example is the interlaboratory comparison on high-throughput sequencing (HTS) analysis, organized by the EURL Virology. This initiative successfully brought together laboratories from multiple countries to evaluate performance, promote harmonization, and build confidence in HTS-based virus diagnostics. Evaluated HTS tests and lessons learned from such interlaboratory comparisons will be provided to EPPO, for inclusion in EPPO Diagnostic Standards and databases.

Keywords: Proficiency testing, interlaboratory comparison, ISO/IEC 17025, high-throughput sequencing, validation.

Pr43

Session: Initiatives for improving plant health cooperation in diagnostics

Strategic pest prioritization and diagnostic synergies across borders in Luxembourg

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This presentation highlights Luxembourg's strategy for prioritizing plant pests and advancing diagnostic capabilities through collaborative efforts. Such a national strategy is mostly based key crops by cultivated area and a thorough review of EU-regulated pests affecting them, to support legislative compliance, assist the National Reference Laboratory and authorities in optimizing analysis, monitoring, and diagnostic methods. It examines how national prioritization guides surveillance and response measures, while emphasizing the value of partnerships, both within the country and across borders, in reinforcing diagnostic precision and resilience. Particular focus is placed on cooperation with laboratories and NPPOs, illustrating how integrated workflows and shared expertise contribute to more effective plant health management. Examples illustrating the pest prioritisation model developed for Luxembourg will be given, including collaborative diagnostic initiatives.

Keywords: Plant pest prioritisation, agriculture, surveillance strategies, diagnostic networks, plant health.

Pr44

Session: Initiatives for improving plant health cooperation in diagnostics

Towards Safer Seeds: The Changing Landscape of Seed Health Testing

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The main staple foods and a variety of plant products that are consumed around the world share the same origin: a seed. More than ever, seeds are globetrotters: they are moved across the world for different goals (e.g. crop production, breeding, seed multiplication, commercialization, research trials). There are a huge number of seeds traded each year: 7 660 613 metric tons exported in 2022 for a value of 16 230 million USD. In such a dynamic economic sector, circulating safe seeds is therefore crucial to secure plant health. However, introductions of pests in new countries worldwide due to infested seeds were recorded as early as the 1880s, confirming that seeds can be a pathway for pest dissemination. In today's rapidly changing world, plant disease emergences can occur everywhere, for multiple reasons - climate change, global trade, or evolving agricultural practices. Disease emergences require rapid reaction time. Therefore, there is a need for a faster delivery of seed health knowledge: which pests are associated with which seeds, and which detection method is most appropriate? The seed sector has made impressive progresses in improving seed health quality. The presentation will offer a concise overview of seed health testing, highlighting key historical and technical developments with an emphasize on several international initiatives for improving seed (and plant) health designed to benefit a large scope of users, including technical staff, researchers, students and teachers, policymakers. Such initiatives include the publication by the International Seed Testing Association (ISTA) of curated lists of seed-borne pests, detection methods and high-quality pest images.

Keywords: seed-borne pathogens, seed transmission, detection, pests, seeds.

Abstracts of posters

Session 1: Development and validation of tests

Po01

Session: Development and validation of tests

Validation of a Commercial Diagnostic Kit for the Reliable Detection of Hop Latent Viroid - Challenges, Opportunities, and Standardized Approaches

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Plant pathogens threaten global agriculture, making rapid and reliable diagnostics essential. Commercial kits are essential for reliable diagnostics, and they need to perform consistently across varying conditions, settings, and laboratories. As new pathogens and variants emerge and standards evolve, diagnostic tests must be continuously adapted. Here, we present the validation of the LOEWE REALTIME® PCR kit for detecting Hop Latent Viroid (HLVd, *Cocadviroid latenshumuli*) as an illustrative case. Although HLVd often shows no visible symptoms, its impact on hop yield and quality makes it a pathogen of economic concern. Its latent nature makes early detection difficult, and its stability and ease of transmission via plant material make it challenging to control. Therefore, reliable, standardized tests with robust validation data are crucial for the timely identification and effective containment of HLVd.

Keywords: HLVd, plant diagnostics, real-time PCR validation, latent infections, hop protection.

Po02

Session: Development and validation of tests

Optimising fast polymerases for the rapid detection of the *Ralstonia solanacearum* species complex by real-time PCR

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The *Ralstonia solanacearum* species complex (RSSC) is a major threat to agricultural productivity worldwide due to its pathogenicity on a wide range of crops, including potato. Early and accurate detection is essential for effective disease management and containment. Conventional real-time PCR workflows, depending on the amplification programme, instrument and polymerase, can take around 1 hour and 40 minutes, prolonging the diagnostic process. Rapid results, however, are particularly critical in trade, where delays can disrupt smooth trade flows. We evaluated nine commercially available mastermixes for their ability to quickly and reliably detect the *Ralstonia solanacearum* species complex in potato extracts. Performance was assessed with two established tests: the Vreeburg et al. (2016) test for total *Ralstonia solanacearum* species complex and the Weller et al. (2000) B2 test specific to *R. solanacearum*. Potato extracts were spiked with low concentrations of *Ralstonia solanacearum* species complex, while negative controls contained potato extracts only. Pure DNA from *R. solanacearum* and *R. pseudosolanacearum* served as positive controls. Analyses were carried out on ABI QuantStudio7 Pro equipment, using the fastest temperature ramping available and shortest hold times recommended for each mastermix. Metrics included total cycling time, sensitivity, specificity, robustness in plant matrices, and intra- and inter-run reproducibility. Several fast polymerase formulations maintained amplification efficiency, specificity, and reproducibility comparable to conventional protocols, while significantly reducing cycling times. This head-to-head evaluation provides practical guidance on combining established tests with fast polymerase mastermixes and optimised cycling parameters. The results support plant health laboratories in reducing turnaround times for routine surveillance and trade-related testing.

Keywords: *Ralstonia solanacearum* species complex, potato, early detection, plant health diagnostics, real-time PCR.

Po03

Session: Development and validation of tests

Development and Validation of a Protocol to Detect *Pseudomonas syringae* pv. *syringae* in Bean (*Phaseolus vulgaris*) Seed

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The bacterium *Pseudomonas syringae* pv. *syringae* is globally distributed and exhibits a high genetic diversity. It has the broadest host range (over 177 hosts described) of *Pseudomonas syringae* pathovars. The observable brown spot disease on bean is only caused by certain isolates of *P. syringae* pv. *syringae*, which is a seed transmitted pathogen. Infected seeds contribute to the long-distance dissemination of the pathogen. Therefore, the use of healthy bean seed is a key strategy to manage this disease and to prevent its introduction into new areas. A detection method, based on dilution plating, is available (Mohan and Schaad, 1987). However, no specific molecular tools have been established to date. An International Seed Health Initiative (ISHI) project was launched to internationally develop and validate a protocol to detect *P. syringae* pv. *syringae* in bean seed. The protocol includes a dilution plating detection test, followed by a real-time PCR identification test of suspect isolates and a pathogenicity confirmation test. Development of a real-time PCR identification test is challenging due to the broad host range of *P. syringae* pv. *syringae* and the need to discriminate *P. syringae* pv. *syringae* from closely related species and pathovars on bean (pathogenic and epiphytic). Internal projects in seed companies (Rijk Zwaan) and in GEVES led to the development of four real-time PCR tests targeting virulence genes (AvrPto and HopAB1), the gene coding for a Sugar-Binding-Protein (SBP) and the gene peptidase M60. Within this project, these tests were compared on a collection of 81 isolates by two seed companies. Results were reproducible as both companies found similar results for a given primer set. However, 10% of the isolates showed different results among the different real-time PCR tests. To better understand the conflicting results, 23 isolates were further characterized using MALDI-TOF Mass Spectrometry biotyping, sequencing of a housekeeping gene (cts) and pathogenicity testing with three different protocols. Based on the results, the primer set targeting the HopAB1 gene and the one targeting the Peptidase gene were selected for the real-time PCR, along with an internal amplification control. Among the three pathogenicity tests, the test on a detached leaf was selected. The sensitivity, specificity and repeatability of the developed protocol remains to be validated, according to ISHI guidelines, and a comparative test will be organized to assess the reproducibility of the method.

Keywords: Brown spot disease, *Pseudomonas syringae* pv. *syringae*, bean, detection, seed.

Po04

Session: Development and validation of tests

HTS on RNA pools to optimize PCR tests

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Po05

Session: Development and validation of tests

High-value diagnostics with genus-level field tests

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Impact of detection in the field or greenhouse is critical for early identification and mitigation of high consequence plant pathogens. These initial tests are the foundation of a preventive disease management program. For these tests to be effective, they must be easy enough that growers or inspectors will use them, sensitive enough to detect an infected plant, and have broad enough specificity to identify all isolates of the target. Agdia's line of generic field tests, serological and molecular, are used to identify dozens or hundreds of species in less than 30 minutes. This expanding suite of point-of-care tests now includes: *Pseudomonas, Xanthomonas, Dickeya, Begomoviruses, Potyviruses*, and others. By screening for pathogens at the genus-level, analysts can immediately identify how to control the spread of the pathogen while confirmatory laboratories can follow-up with information on the exact species.

Keywords: *Pseudomonas*, *Xanthomonas*, *Dickeya*, *Begomovirus*, *Potyvirus*.

Session 2: Advances in diagnostic technologies used in the field and in the laboratory

Po06

Session: Advances in diagnostic technologies used in the field and in the laboratory

Use of metabarcoding for the detection of *Phytophthora* baited on leaves put in streams near ornamental nurseries

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Exotic *Phytophthora* species pose a serious threat to forests in Europe. These pathogens produce motile zoospores in water. Infection most often occurs at the level of fine roots. Several species of *Phytophthora* can infect forest and ornamental trees, and new species are identified regularly. The international trade in ornamental woody plants plays a key role in the introduction of exotic *Phytophthora* into new geographical areas. Monitoring in ornamental nurseries is therefore an essential step in detecting these new pathogens at an early stage and limiting the risk of their spread in forest environments. Inspections in nurseries are complicated as the range of plants to be checked is large and sometimes unknown for exotic *Phytophthora* species. On the other hand, some infections are discrete, making visual control unreliable. To take these constraints into account, we developed a monitoring strategy based on the selection of nurseries in the vicinity of streams (using QGis tools) and bait leaf tests downstream of these nurseries. The bait leaves are collected after several days in water, and any *Phytophthora* are detected using a metabarcoding method (Illumina MiSeq), followed by bioinformatic analysis of the data (FungiSearch pipeline). The method was validated on a mock community of 26 *Phytophthora* species belonging to eight clades. Twenty-four species were correctly assigned, and the detection limit was ~5 pg/test. The method was then applied to bait leaves from watercourses near seven Belgian ornamental nurseries. Monitoring was conducted over 2 years (2023 and 2024). Eighteen *Phytophthora* species were detected, including species never previously reported in Belgium. A comparison with isolation on PARP-CA medium highlighted the higher efficiency of the molecular method. As the analysis of the data does not require any significant bioinformatics skills and can be conducted on a desktop computer, our method could be used as a screening test in monitoring studies.

Keywords: Early detection, FungiSearch, high throughput sequencing, *Phytophthora*, surveillance.

Po07

Session: Advances in diagnostic technologies used in the field and in the laboratory

Smart Diagnostics for Plant Health: From Electronic Noses to On-Site Molecular Detection

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Advances in pest diagnostics are reshaping plant health surveillance by enabling rapid, field-based detection combined with laboratory precision. New isothermal amplification techniques, such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), now allow on-site molecular detection of plant-parasitic nematodes and other pathogens. These methods operate at constant temperature, require minimal instrumentation, and deliver highly specific results within minutes, representing a breakthrough in portable molecular diagnostics. In parallel, the European project PurPest is pioneering volatile organic compound (VOC)-based detection through electronic-nose (e-nose) systems that recognize pest-specific VOC profiles emitted by infested plants or substrates. This non-invasive, real-time technology complements molecular tools by providing fast, field-deployable prescreening for pest activity. The combination of e-nose sensing and isothermal molecular tests defines a new generation of integrated diagnostic platforms. Together, they enhance early-warning capacity, support precision pest management, and contribute to the European Green Deal goals of sustainable, low-impact agriculture.

Keywords: early detection, field diagnostics; isothermal amplification, nematodes, volatile organic compounds.

Po08

Session: Advances in diagnostic technologies used in the field and in the laboratory

DISRUPP: An Innovative Strategy for Field Health Surveillance Using High-Throughput Imaging and Sequencing to Reduce Plant Protection Product Use

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Modern agriculture faces a major challenge: feeding a growing global population while drastically reducing the use of plant protection products, in line with environmental objectives. The intensive use of these products has raised health and ecological concerns, such as pest resistance and environmental contamination. It is therefore urgent to develop faster, more accurate, and more targeted in-field disease diagnostic methods, which are also essential for organic farming which prohibits synthetic chemical inputs. DISRUPP project, launched in 2025 and financed by FranceAgriMer, aims to address this issue by offering an automated and comprehensive plant health monitoring system. Its innovation lies in the combination of two cutting-edge technologies: satellite and drone imaging for early detection of potential symptoms, and third-generation Oxford Nanopore high-throughput sequencing for rapid and accurate diagnosis. Imaging allows regular monitoring to identify at-risk areas. This detection triggers sampling which will then be subsequently analyzed using sequencing methods capable of exhaustively identifying pathogens through the analysis of long DNA fragments, without the biases inherent to PCR. The project also includes the development of a simplified analysis platform enabling fast diagnosis. The initial trials focused on various plant species, including cereal crops, legumes, tree crops, bulb plants, and grapevine, cultivated under both organic or conventional farming systems. At each representative vegetative stage of the crops, samples were collected for DNA extraction and sequencing, and a drone flight was conducted to enable a comparative analysis of the symptoms observed in the field. The potential outcomes of the project are multiple and significant. DISRUPP will enable farmers to obtain near real-time information on the health status of their crops, facilitating targeted interventions and reducing the preventive use of plant protection products. This will help lower agriculture's environmental footprint and meet the targets of environmental action plans.

Keywords: Crop health monitoring, Fast diagnosis, Imaging, Nanopore sequencing.

Po09

Session: Advances in diagnostic technologies used in the field and in the laboratory

Innovating plant health diagnostics: Nanopore sequencing for quarantine bacterial pathogens

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Accurate detection and identification of regulated pests directly from plant material, including asymptomatic samples, is essential for effective phytosanitary surveillance. Most diagnostic tests for phytopathogenic bacteria rely on molecular methods such as conventional PCR, real-time PCR, and LAMP, while identification involves MLST/MLSA approaches based on housekeeping gene sequencing. However, these tests present challenges when applied for the detection of asymptomatic plant material or for the specific identification of pathovars/subspecies. To address this challenge, we developed a diagnostic system based on Nanopore sequencing, using the portable MinION device from Oxford Nanopore Technologies (ONT). Two complementary strategies were evaluated: (i) a preliminary shotgun metagenomic approach for a few time-consuming processing systems and ii) amplicon-Nanopore sequencing, which combines multiplex-PCR amplification of selected housekeeping genes with MONICA®, a custom bioinformatics pipeline tailored to ONT data. Preliminary shotgun metagenomics, carried out on olive samples spiked with *Xylella fastidiosa* subsp. *pauca* using cost-effective ONT Flongle flow cells, yielded promising results, allowing reliable detection at high bacterial concentrations. Nevertheless, improvements are required to increase sensitivity at lower pathogen loads. Amplicon-Nanopore sequencing was applied to priority EU-regulated quarantine pathogens-*Xylella fastidiosa* subspecies, *Xanthomonas citri* pv. *citri* and pv. *aurantifolii*, and *Pantoea stewartii* subsp. *stewartii*. These species include multiple subspecies, pathovars, and sequence types with distinct host ranges and geographic distributions, making high-resolution taxonomic identification essential for guiding appropriate phytosanitary actions. This system was successfully applied to both artificially spiked and naturally infected plant samples, showing high overall sensitivity and specificity, while also highlighting pathogen-dependent strengths and limitations. The overall findings of the study highlight the potential of these sequencing approaches as a portable, reliable, and accessible solution for quarantine and NQP diagnostics and plant health monitoring.

Keywords: Next-generation tools, portable diagnostics, phytosanitary surveillance, point-of-care, bacterial pathogens.

Po10

Session: Advances in diagnostic technologies used in the field and in the laboratory

Introducing automation into the high-throughput sequencing workflow for plant pathogen detection on a laboratory scale: experience at Leibniz Institute DSMZ

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High-throughput sequencing (HTS) has become a cornerstone in plant pathogen diagnostics, supporting applications ranging from routine surveillance to outbreak response. Over the past several years, the Plant Virus Department at DSMZ has implemented HTS in diagnostics and research activities. Leveraging state-of-the-art methodologies, the department has established a comprehensive workflow encompassing sample preparation and nucleic acid extraction, library construction, and sequencing in house. Yet, the scalability of these efforts can be limited by hand-executed preparation workflows, which may introduce variability and constrain throughput. The aim of automated liquid handling platforms is to address these challenges by enabling precise and reproducible sample processing, thereby minimizing operator-dependent variability. In addition, their use is intended to reduce hands-on time and enhance laboratory efficiency, ultimately supporting reliable and scalable workflows. In this perspective, we have evaluated the performance of a compact, liquid-handling platform for semi-automated library preparation into our HTS pipeline. A comparative analysis with the standard, non-automated preparation workflow will be presented and the respective performance discussed.

Keywords: high-throughput sequencing, library preparation, automation, liquid handling.

Po11

Session: Advances in diagnostic technologies used in the field and in the laboratory

SeqDetectVeg: a major project to develop a NGS tools for multi-target detection of bacterial and fungal pathogens transmitted by vegetable seeds

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Seeds enable genetic resources to be disseminated as part of programmes to select, multiply and market varieties throughout the world. At the same time, seeds are also carriers of a diversity of micro-organisms (collectively referred to as the microbiota), which can be beneficial or detrimental to plant health. The availability of accurate and high-throughput methods for identifying seed lots of high sanitary quality is therefore essential for the seed industry. SeqDetectVeg is a collaborative project, between seed companies, academic institutes and the French Variety and Seed Study and Control Group (GEVES), which proposes to use metabarcoding (amplicon sequencing), to develop and validate methods for detecting multiple bacterial and fungal pathogens in seed lots of different vegetable species. This approach has already proved its worth in seed microbiota studies and in pilot projects. Various metabarcoding markers, such as the ITS (Internal Transcribed Spacer rRNA) region and the *gyrB* (DNA gyrase subunit B) gene for fungal and bacterial communities respectively, have been pre-selected for this project.

Keywords: Diagnostic, Vegetable Seeds, Pathogens, Next Generation Sequencing, Metabarcoding.

Po12

Session: Advances in diagnostic technologies used in the field and in the laboratory

Detection of phytoplasma diseases in Rhineland Palatinate

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Phytoplasmas are obligate intracellular parasites of plant phloem tissue and of the insect vectors that are involved in their plant-to-plant transmission. Phytoplasmas are pathogens of agriculturally important plants. In Rhineland Palatinate, the two most acute threats are flavescence dorée of grapevine and syndrome des basses richesses disease (caused by '*Candidatus* Phytoplasma solani' and '*Candidatus* Arsenophonus phytopathogenicus' respectively) on different types of crops. Therefore, controlling these diseases has become a top priority, with the first crucial step being accurate and efficient diagnosis of phytoplasma infections. The first critical step in routine diagnosis is DNA extraction, which can be affected by the type of crop and nature of material (leaf material, wood, roots), and the number of samples to be tested. Different DNA extraction protocols are currently in use, including CTAB extractions (for low number of samples), column-based extractions, or automated DNA extractions (for high number of samples). The resulting DNA extracts are then subjected to conventional PCR, nested PCR, real-time PCR, or LAMP when available, according to current EPPO Standards. In most of the cases, a high degree of accordance was observed between the different methods.

Keywords: phytoplasma, DNA extraction, molecular diagnosis.

Po13

Session: Advances in diagnostic technologies used in the field and in the laboratory

Evaluation and Application of HTS in Plant Health Diagnostics: A Comparative Study of Second and Third Generation Sequencing (Illumina vs. ONT)

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High-throughput sequencing (HTS) has become a key tool in phytopathological diagnostics, and its use is recognized in EPPO Standard (PM 7/151). However, the current PM 7 Standards do not yet include specific details on library preparation methodologies or bioinformatic procedures, which hampers transferability, reproducibility, and standardization of analyses. In this context, and within the framework of the EPPO Jens-Georg Unger Plant Health Fellowship, a project was developed in collaboration with the National Reference Laboratories for Bacteriology and Virology in France and Spain (IVIA, ANSES) to comparatively assess Illumina technology, considered the reference standard, and Oxford Nanopore Technologies (ONT), a third-generation platform characterized by its portability and real-time data generation. Rapid DNA libraries were prepared (DNA Prep for Illumina and Rapid Sequencing Kit for ONT), together with the implementation of an ad hoc bioinformatic pipeline, applicable to Illumina, ONT, and ONT-Rolling Circle Amplification (RCA) data, designed to ensure reproducibility and repeatability of analyses. An initial comparison with two begomovirus pathosystems, Tomato leaf curl New Delhi virus (*Begomovirus solanumdelhiense*) and sweet potato leaf curl virus (*Begomovirus ipomoeae*), showed that genomes reconstructed with both approaches displayed >99% identity, with no relevant differences. Based on these results, the ONT-RCA approach was selected for validation according to PM 7/98, due to its suitability as a portable technology transferable to diagnostics. This strategy is currently being validated with a panel of 26 viral species from the genera *Begomovirus, Becurtovirus, Nanovirus, Curtovirus, Badnavirus,* and one additional species from the family Geminiviridae, covering both horticultural crops and woody and fruit species of diverse origins. In addition, the sensitivity of the ONT-RCA method is being evaluated in comparison with previously validated PCRs, in order to establish its limits of detection and diagnostic robustness. Preliminary results confirm that ONT-RCA, combined with a standardized pipeline, represents a rapid, reliable, and reproducible approach with strong potential for implementation in reference laboratories and inspection points, contributing to the future development of EPPO-specific Standards for HTS in phytopathological diagnostics.

Keywords: Identification, HTS, Nanopore, RCA.

Po14

Session: Advances in diagnostic technologies used in the field and in the laboratory

Isothermal and Digital PCR-Based approaches for *Xylella fastidiosa* detection in plant extracts

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Xylella fastidiosa is a quarantine pest causing devastating plant diseases such as Olive Quick Decline Syndrome, Pierce's disease in grapevine, and almond leaf scorch. Early and accurate detection is critical to prevent the establishment and spread of this bacterial pathogen in areas with favourable conditions. At EU level, mandatory surveillance programs including visual inspections and laboratory testing are now enforced as phytosanitary measures to protect major mediterranean crops. While real-time PCR tests currently are the gold standard for bacterial detection, significant investments have been made in research into rapid on-site diagnostic tests, mainly Loop-mediated isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA). In the framework of two EU funded projects (FREE@POC - GA 862840; BeXyl - GA 101060593), two diagnostic approaches based on colorimetric LAMP (cLAMP) and RPA were developed for *in-situ* detection in alkaline plant extract prepared from naturally infected *Olea europaea*, *Prunus dulcis* and *Vitis vinifera*. For olive trees thin slices (0.3 - 0.5 mm thick) of semi-hardwood branches were selected (Amoia et al. 2023), while leaf petioles or xylem tissues scraped from de-barked branches or canes were used for almond and grapevine respectively. After crushing the tissue with a hammer in a plastic bag containing an alkaline buffer, the recovered extract, upon neutralization and dilution, was loaded into the cLAMP reactions as described in Amoia et al. (2023) or added to the RPA reactions following the TwistAmp Basic kit instructions (TwistDx, GB) and an incubation at 37°C for 20 min. An aliquot of the same extract was also tested in droplet digital PCR for the quantification of the bacterium. The primer pairs previously designed by Harper et al. (2010) or newly designed and targeting the *rimM* gene of *X. fastidiosa* were used to set up the three tests. Overall, 282 olive, 22 grapevine and 32 almond samples infected, respectively, by isolates of the *X. fastidiosa* subsp. *pauca, fastidiosa* and *multiplex* were used for the validation. The three tests showed high specificity regardless of the host plant, and the use of this extract did not produce false positives or impact the PCR efficiency since all samples testing negative with real-time PCR gave negative reactions with the newly developed tests (ddPCR, cLAMP and RPA). Xylem tissue was confirmed to be the preferred tissue for *X. fastidiosa* diagnosis in almond and grapevine. The limit of detection reached 100 CFU/mL when using both ddPCR and cLAMP and 10^3 CFU/mL using RPA. The results gathered showed that these plant extracts are suitable for the detection of *X. fastidiosa* in olive samples. Indeed, both cLAMP and RPA offer the possibility to work on site, requiring limited instruments and facilities; ddPCR can be used on the same extracts to confirm the results obtained with the initial on-site diagnostic tests.

Keywords: *Xylella fastidiosa*, alkaline extracts, colorimetric LAMP, RPA, ddPCR.

Session 3: Diagnostics of emerging & re-emerging pests

Po15

Session: Diagnostics of emerging & re-emerging pests

Beyond visual Inspection: validation of an eDNA-based method for the early detection of *Toumeyella parvicornis*

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Field-based phytosanitary inspections often face significant limitations, particularly during the early stages of pest establishment when populations are low and symptoms are either faint or entirely absent. A clear example is *Toumeyella parvicornis* (pine tortoise scale), whose detection on large pine trees can be extremely challenging. Early infestations typically lack visible signs such as needle or branch blackening, leading to a high risk of underestimation through visual surveys alone. To address these challenges, we developed and validated an innovative diagnostic approach based on environmental DNA (eDNA) analysis. The method targets molecular traces left in the honeydew excreted by the insect and deposited on foliage and surfaces below the tree canopy. Sampling is performed using absorbent swabs, followed by a SYBR Green real-time PCR test targeting a 197 bp fragment of the mitochondrial ND1 gene. The test was validated according to EPPO Standard PM 7/98 (6) and demonstrated high specificity, sensitivity, and accuracy. It successfully detects all known haplotypes of *T. parvicornis*, from both its native range and introduced areas, without cross-reactivity with other *Toumeyella* species or with non-target honeydew-producing insects sharing the same ecological niche. The method was tested over two consecutive years in a *T. parvicornis*-infested area in Italy, alongside conventional visual monitoring, and was also validated under semi-controlled field conditions. The results obtained provide useful operational guidance for phytosanitary technicians, equipping them to effectively integrate the method into conventional monitoring practices. Integrating eDNA analysis with traditional inspection protocols offers a powerful tool for early detection, improving the timeliness and precision of phytosanitary actions against *T. parvicornis* and supporting more targeted and effective management strategies.

Keywords: Environmental DNA (eDNA), *Toumeyella parvicornis,* Early detection, Real-time PCR, Phytosanitary monitoring.

Po16

Session: Diagnostics of emerging & re-emerging pests

Dual threat from beetle-fungus-mutualism: The case of *Euwallacea fornicatus sensu lato* and *Neocosmospora* sp. in North Rhine-Westfalia

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The EU-wide regulated and EPPO A2-listed ambrosia beetle *Euwallacea fornicatus sensu lato* has been found for the first time in the German federal state North Rhine-Westfalia (NRW) in 2024. Both larvae and adults have been recorded in the infested greenhouse on various woody plants such as *Adansonia digitata*, *Ficus religiosa*, *Sterculia nobilis* and *Triplaris americana*. This beetle species complex origins from South-East Asia and infests more than 400 host plant species. *Euwallacea fornicatus sensu lato* beetles can transmit *Neocosmospora* fungi to the infested host plants causing wilting and dieback. Recently, novel fungi of the *Neocosmospora* genus have been isolated from infested plants and beetles in NRW. Both beetles and fungi pose a dual threat to local host plants. Findings of diagnostics based on infected host plant material as well as specimens of *Euwallacea fornicatus sensu lato* and *Neocosmospora* sp. in NRW are presented.

Keywords: Germany, Scolytinae, Nectriaceae, mutualism, polyphagous pests.

Po17

Session: Diagnostics of emerging & re-emerging pests

First signs and future threats: monitoring phytoplasma infections and vectors in Dutch seed potatoes

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Following the fast and devastating emergence of '*Candidatus* Phytoplasma solani' (PHYPSO) and '*Candidatus* Arsenophonus phytopathogenicus' (ARSEPH) in several European countries, the Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes (NAK) has initiated monitoring activities for these emerging pests and their vectors in the Netherlands. Monitoring activities include the training of field inspectors to recognize symptoms, supported by literature and photos, and subsequently the identification of symptomatic plants in the field and molecular testing. So far, we were able to confirm infections with '*Candidatus* Phytoplasma asteris' and '*Candidatus* Phytoplasma fragariae', the latter reported for the first time in Dutch seed potatoes. However, '*Ca.* Arsenophonus phytopathogenicus' and the causal agent of stolbur, '*Ca.* Phytoplasma solani' have not yet been detected in Dutch seed potatoes. To assess vector occurrence, prevalence of cicadas is monitored in ~40 aphid traps across seed potato regions in the Netherlands. The main vectors, *Pentastiridius leporinus* and *Hyalesthes obsoletus*, are generally rare in the Netherlands, but the capture of one *H. obsoletus* demonstrates the efficacy of this approach and confirms the presence of the vector in Dutch potato fields. Ongoing work focuses on validating diagnostic tests and conducting experiments to support evidence-based monitoring and policy development.

Keywords: *'Candidatus* Phytoplasma solani*'* (PHYPSO), *'Candidatus* Arsenophonus phytopathogenicus*'* (ARSEPH), *Solanum tuberosum* (SOLTU), phytoplasma generic detection (EPPO PM 7/133); vector monitoring (*Pentastiridius leporinus*, *Hyalesthes obsoletus*).

Po18

Session: Diagnostics of emerging & re-emerging pests

First Records of *Meloidogyne enterolobii* in Italy and the Diagnostic Workflow of the National Network

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The international trade of plants and plant products increases the risk of introducing harmful organisms, including quarantine nematodes. Among them, *Meloidogyne enterolobii* is an emerging threat due to its high invasive potential and significant economic and environmental impact. In Italy, the first detection occurred in 2023 on *Ficus microcarpa*, followed by additional interceptions in subsequent years. The management of these cases involved the network of official Italian laboratories, which performed first-level analyses using morphological and molecular methods. These diagnoses were subsequently confirmed by the National Reference Laboratory (NRL) in Florence, through second-level testing and advanced analyses, ensuring both reliability and compliance with European standards. This multilayered approach enabled the timely validation of findings and the prompt activation of phytosanitary measures for nematode containment. The Italian cases highlight the importance of a structured and coordinated diagnostic network, integrating surveillance, preliminary screening, and specialized confirmation, in support of European strategies to prevent the introduction and spread of high-impact quarantine pests.

Keywords: diagnosis, *Ficus microcarpa*, National Reference Laboratory, quarantine nematodes, Root-knot nematode.

Po19

Session: Diagnostics of emerging & re-emerging pests

Tests for *Globodera rostochiensis* and *Globodera pallida*

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Potato cyst nematodes, primarily *Globodera rostochiensis* and *G. pallida*, are highly specialized soil borne parasites that pose a significant threat to global potato production. These microscopic organisms infect the roots of potato and other Solanaceous plants, leading to reduced plant vigour and substantial yield losses. The life cycle of potato cyst nematodes is completed within approximately 38-48 days under suitable conditions, making them persistent and difficult to eradicate. Symptoms of potato cyst nematodes infestation include stunted growth, chlorosis, wilting, and the formation of small yellow or brown cysts on potato roots. As the nematodes spread primarily through contaminated soil, machinery, and plant material, strict hygiene and testing are critical for prevention. The absence of the potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida* in Albania is assessed through a phytosanitary monitoring programme based on laboratory analyses of soil samples. Within this framework, 700 soil samples collected from potato cultivation fields across the country were analysed. The results of the analyses did not reveal the presence of these quarantine pests in any of the examined samples. Based on the survey data, there is currently no evidence of their presence in the monitored areas of Albania. However, constant testing and the implementation of integrated management and biosecurity measures remains essential to prevent the establishment and spread of these pests, given that the country receives many imports of host plants and their seeds.

Keywords: *Globodera*, quarantine, surveillance, diagnostics, biosecurity.

Po20

Session: Diagnostics of emerging & re-emerging pests

Impact of tomato brown rugose fruit virus (ToBRFV) on infection dynamics, yield reduction, and economic impact in Austrian tomato production systems

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The tomato brown rugose fruit virus (ToBRFV) was first detected at tomato production sites in Austria in 2021. During the growing seasons of 2023 and 2024, infected locations were monitored to track the progression of the virus in tomato crops, including non-resistant, tolerant, and resistant varieties. Findings indicate that ToBRFV infection levels can be effectively kept low within isolated greenhouse compartments, provided that tomato varieties grown within this units possess at least tolerance to the virus. This containment effect persists even when adjacent compartments within the same production facility exhibit high infection levels. Data on production losses and associated additional costs resulting from ToBRFV infections were systematically collected from affected tomato production sites. During the initial outbreak years, tomato producers experienced substantial yield reductions. Yield losses varied significantly depending on the tomato variety, with some cultivars exhibiting reductions of up to 400 000 kg per hectare. The outbreak of a quarantine pest resulted in substantial additional costs for production farms, impacting a broad spectrum of operational areas. These financial burdens were not limited to the outbreak year but persisted in subsequent years, reflecting the long-term economic implications of the infection.

Keywords: tomato greenhouse production, ToBRFV, infection progress, economic impact of virus infections.

Session 4: Plant Health diagnostics from a broader perspective

Po21

Session: Plant Health diagnostics from a broader perspective

Improving Culture Confirmation of *Pantoea stewartii* subsp. *stewartii* in Slovenia through Systematic Subsampling

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Pantoea stewartii subsp. *stewartii*, the causal agent of Stewart's wilt of maize (*Zea mays* L.), can cause significant economic losses. The pathogen was first confirmed in Slovenia in 2018 in two maize samples. By the end of 2024, a further 19 maize samples were confirmed through molecular diagnostics and culture-based isolation. During this period, 19 additional samples tested positive using two independent molecular tests but could not be confirmed by isolation. In most of these cases, abundant saprophytes and/or other bacteria were present, some of which morphologically resembled the target bacterium, complicating reliable identification. To address this, we introduced a systematic subsampling approach targeting diverse symptomatic tissues. Parallel molecular and culture-based testing enabled assessment of symptom type, bacterial concentration, and colony abundance. While this method improved the recovery of *P. stewartii* subsp. *stewartii*, it also increased workload and costs, especially in the early stages when diagnostic expertise was still being developed. These hidden costs are often underestimated by funders and highlight the gap between molecular screening, which may rely on a single sample per plant, and the more demanding requirements of culture-based confirmation. This strategy nevertheless improved the recovery of *P. stewartii* subsp. *stewartii* and supports more reliable culture confirmation.

Keywords: *Pantoea stewartii* subsp. *stewartii*, Stewart's wilt of maize, Subsampling strategy, Culture-based isolation, Molecular diagnostics.

Po22

Session: Plant Health diagnostics from a broader perspective

Seed-Transmitted Pathogens in Tomato and Pepper: Toward an Improved and Reliable Detection Method Using seed extract real-time PCR

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Seed-transmitted bacterial pathogens can cause severe crop losses and pose a significant threat to agricultural productivity and international seed trade. In tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*), notable pathogens include *Clavibacter michiganensis*, which primarily affects tomato, and several *Xanthomonas species* (*X. euvesicatoria*, *X. gardneri*, *X. perforans*, and *X. vesicatoria*), which impact both crops. Effective seed health testing is critical to prevent the dissemination of these regulated non-of-quarantine pests across countries. The current official French detection method for *C. michiganensis* in tomato seeds relies on bacterial isolation, followed by molecular confirmation through real-time PCR and a pathogenicity test. A similar workflow is applied for detecting *Xanthomonas* spp. in both tomato and pepper seeds. While reliable, this protocol is labour-intensive and time-consuming, with a turnaround time of up to 12 days. At the international level, the ISHI method developed by the International Seed Federation (ISF) offers a faster alternative by incorporating a direct real-time PCR screening from seed extracts without prior isolation. This approach enables rapid screening and clearance of negative seed lots, with results available within 48 hours. However, its sensitivity is significantly reduced when applied to seeds treated with sodium hypochlorite, a common disinfection practice. This collaborative project aims to develop an enhanced seed extract real-time PCR test for the detection of *C. michiganensis* and *Xanthomonas* spp. that is compatible with hypochlorite-treated seeds, maintaining equivalent sensitivity and specificity to that observed with untreated seeds. A further objective is to optimize and harmonize the maceration and DNA extraction steps to enable the simultaneous detection of all five targeted pathogens from a single seed extract. Based on recent data from GEVES (2022-2023), approximately 95% of tested seed lots are free of these pathogens. Implementing a validated seed extract real-time PCR approach would therefore allow for a substantial reduction in testing time and laboratory workload, while preserving diagnostic accuracy. For this project, the production of natural contaminated seeds by *C. michiganensis* was performed followed by the disinfection of seeds. The results for three different extraction methods and their impact on sensibility will also be presented.

Keywords: Seed-transmitted, tomato, seed extract, sensibility, seed treatment.

Po23

Session: Plant Health diagnostics from a broader perspective

PHID-Coleo project results: Identification tools for wood boring beetles in plant health inspections.

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Wood damaging beetles that might be introduced via global trade are a major threat to plant health. To allow early detection during border control and plant inspections new methods for species identification of non-indigenous beetles on wood packaging material as well as on living plants have been tested and adapted. The solution for integrative diagnostics are classical entomology (a pictured identification key), molecular biology (LAMP, Metabarcoding) and new digital methods (smartphone application with image recognition). Two projects focussed on Cerambycidae, Bostrichidae, Scolytinae and Buprestidae, e.g. Asian longhorn beetle *Anoplophora glabripennis*, red neck longhorn beetle *Aromia bungii*, emerald ash borer *Agrilus planipennis* and the walnut twig beetle *Pityophthorus juglandis*. Within this research project, a large illustrated catalogue of potentially wood harming beetle species has been developed for plant health purposes and is free to be used by diagnostic laboratories in plant protection services.

Keywords: insect diagnostics, integrative taxonomy, metabarcoding, identification key, automated identification.

Po24

Session: Plant Health diagnostics from a broader perspective

MALDI-TOF MS profiling of maize isolation plates: from background flora to overlooked pathogens

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Isolation of *Pantoea stewartii* subsp. *stewartii* from maize and other host plants is routinely performed on King's B medium, yet this process is often complicated by abundant background flora whose colonies can mimic the target pathogen. To document and better manage this challenge, we systematically applied MALDI-TOF MS to identify colonies recovered during routine isolations. The resulting dataset, which also serves as validation data for MALDI-TOF in this context, included both expected pathogens and recurring contaminants. Several taxa were repeatedly encountered and shown to complicate diagnostics, including *Sphingomonas*, *Curtobacterium*, *Microbacterium Agrobacterium* and diverse *Pantoea* species. *Pantoea ananatis* and *Microbacterium* spp., which closely resemble *Pantoea stewartii* in colony appearance were particularly challenging. This work provides a practical reference for improving test specificity, developing realistic reference materials, and training diagnosticians. As an outcome, we plan to compile a photo gallery linking colony images, both to support colony selection and to highlight overlooked pathogens such as the frequent occurrence of *Pantoea ananatis*.

Keywords: maize diagnostics, MALDI-TOF MS, colony selection, background flora, *Pantoea stewartii*, *Pantoea ananatis.*

Po25

Session: Plant Health diagnostics from a broader perspective

Reassessing Historical Presence of *Dothistroma pini* and *Dothistroma septosporum* in Central and Southern Europe Using Herbarium Specimens

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Herbarium collections serve as a valuable reference archive of plant specimens, precisely documented in both time and space. In addition to information about the collected plants themselves, the historical DNA preserved in these samples offers unique insights into the spread and genetic evolution of plant pathogens over time. Advances in molecular identification techniques now allow for the reanalysis of specimens that were previously classified using only morphological characteristics. For example, *Dothistroma pini* and *D. septosporum*, the fungal pathogens responsible for Dothistroma needle blight, were only recognized as two separate cryptic species in 2004. Moreover, Dothistroma needle blight symptoms can resemble those of other pine needle diseases, including brown spot needle blight, caused by *Lecanosticta acicola*. As earlier identifications based solely on morphology may therefore be unreliable, we performed a molecular re-examination of a selection of specimens stored in collections from Austria, Czechia, Croatia, Slovakia and Slovenia, which have been previously diagnosed with Dothistroma needle blight or brown spot needle blight symptoms. Symptomatic specimens collected as far back as 1978 were reanalysed. Using a specific real-time polymerase chain reaction, the fungal pathogens associated with the symptoms were successfully identified in almost all of them, shedding light on the historical presence of *D. pini* and *D. septosporum* in parts of Central and Southern Europe.

Keywords: BSNB, DNB, *Dothistroma pini*, *Dothistroma septosporum*, *Lecanosticta acicola*, historical DNA, real-time PCR, molecular identification.

Po26

Session: Plant Health diagnostics from a broader perspective

BeetClinic at Strube D&S (RAGT Group): diagnostics of pests and diseases in sugar beet and other crops

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The correct diagnosis of plant diseases and pests plays a critical role that can impact crop yield and quality. Accurate diagnosis involves the detection of pathogens such as fungi, bacteria, viruses and nematodes with different microscopic and molecular methods. At our diagnostic laboratory, different types of samples including soil and plant material from Germany and other countries have been tested since 2021. Our data shows that the field observation of different sugar beet pests and diseases and the respectively demands for diagnosis changes throughout the years. Over the last two years, the bacteria causing Syndrome Basses Richesses (‘*Candidatus* Arsenophonus phytopathogenicus’) and Stolbur (‘*Candidatus* Phytoplasma solani’) are the most common pathogens detected in sugar beet samples analysed in our laboratory. This information is crucial for helping those running breeding programmes and farmers with correct field management.

Keywords: sugar beet, plant disease diagnostics, disease surveillance, syndrome basses richesses.

Session 5: Initiatives for improving plant health cooperation in diagnostics

Po27

Session: Initiatives for improving plant health cooperation in diagnostics

Improving *Xylella fastidiosa* protocols by interlaboratory comparisons

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The European Union reference laboratory for pests on plants on bacteria (EURL-BAC) is a consortium of four laboratories; ILVO (Belgium), CREA-DC (Italy), NIB (Slovenia) and NVWA-NIVIP (the Netherlands). The EURL-BAC has organized different interlaboratory comparisons on the detection and sub-species determination of *Xylella fastidiosa*. The proficiency test on *X. fastidiosa* subspecies determination has led to new insights in the specificity of two molecular tests; the conventional PCR by Hernandez-Martinez et al. (2006) and the real-time PCR by Hodgetts et al. (2021). These findings have led to the corrigendum on EPPO Diagnostic Standard PM 7/024 (5) published in 2024. The findings that have led to this corrigendum are presented.

Keywords: *Xylella fastidiosa*, PCR, specificity.

Po28

Session: Initiatives for improving plant health cooperation in diagnostics

Spanning the Gap: Agdia's role as a diagnostic company connecting public and private agencies globally

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For over forty years, Agdia has connected the plant health community by bridging gaps between research scientists, regulatory agencies and the producers they serve. As a diagnostics partner, Agdia provides the rapid response and throughput necessary to address emerging threats from invasive pests and pathogens to outbreaks of resistance breaking races and strains. As a commercialization partner, Agdia leverages its international distribution and quality management system to ensure a new test can have the farthest reach for the longest time possible. Our innovation remains focused on the end users and their often-practical needs such as storage and simplicity. Now in its third generation, Agdia is refining the latest molecular detection techniques to once again lower barriers to test adoption and expand their use on a global scale.

Keywords: commercial, molecular, distribution, detection, response.

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Session: Initiatives for improving plant health cooperation in diagnostics

Improving phytosanitary testing capacity in the reference laboratory in the Republic of Moldova within the Moldovan-Czech project

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Strengthening collaboration among diagnostic laboratories is a key pillar in safeguarding plant health at both regional and global levels. Through joint efforts, laboratories can improve the precision and efficiency of detecting pests and diseases, exchange vital information and tools, and respond more swiftly to new and evolving threats. This type of collaboration - whether international, - or in partnership with National Plant Protection Organizations - promotes the standardization of procedures, encourages the adoption of new technologies, and strengthens the overall capacity to protect plant health through coordinated and timely actions. Recognizing the value of this collaborative approach, the Central Institute for Supervising and Testing in Agriculture of the Czech Republic (ÚKZÚZ), with funding from the Czech Government under its Development Cooperation program, launched a major project in 2024 entitled “Improving Control and Testing Capacity in the Phytosanitary Field within the Republic of Moldova”. This initiative aims to strengthen Moldova’s phytosanitary system through multiple components, including the provision of modern laboratory equipment and advanced information systems, targeted training for laboratory personnel and inspectors from the National Food Safety Agency (ANSA), and support for the harmonization of national legislation with European Union standards. Such initiatives are of paramount importance for non-EU countries such as the Republic of Moldova, as they facilitate the alignment of national phytosanitary standards with international best practices, thereby enhancing the country's ability to prevent and manage plant health risks. By meeting EU phytosanitary standards, Moldova is better positioned to expand export opportunities for key agricultural products, such as plums, cherries, grapes, and apples, thereby strengthening its economy and contributing to regional food security. By strengthening diagnostic and regulatory capacities, these projects contribute to improved agricultural productivity, support sustainable development and integration into broader international plant health networks, and foster cooperation and resilience against transboundary plant pest threats.

Keywords: Improving phytosanitary testing capacity, plant health, reference laboratory, collaboration, Moldovan-Czech project.

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Session: Initiatives for improving plant health cooperation in diagnostics

Establishment of the National Reference Laboratory (NRL) at SVA, Sweden

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The Swedish Veterinary Agency (SVA) is currently establishing the National Reference Laboratory (NRL) for plant health diagnostics in Sweden. With this poster, we aim to introduce the newly formed NRL team, present the main activities and priorities during the establishment phase, and highlight opportunities for collaboration with the wider EPPO community. The NRL will strengthen national diagnostic capacity, ensure alignment with European and international standards, and serve as a hub for expertise, training, and collaboration. As part of this process, SVA is implementing EPPO Diagnostic Standards, building networks with other laboratories and stakeholders, and establishing quality management structures to support reliable and timely detection of regulated pests.

Keywords: National Reference Laboratory Sweden, The Swedish Veterinary Agency (SVA), Diagnostic capacity building, Plant Health networking.

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Enhancing Plant Health Diagnostic Proficiency through Interlaboratory Comparisons Based on DNA Barcoding and Reference Collections

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In Slovenia, three official laboratories (the Slovenian Forestry Institute, the Agricultural Institute of Slovenia and the Slovenian Institute of Hop Research and Brewing) form the National Reference Laboratory for fungi and oomycetes, each specializing in different host plant groups. To address the limited availability of formal proficiency tests in plant health diagnostics, we initiated annual small-scale interlaboratory comparisons (ILCs) using DNA barcoding. Since the establishment of the consortium in 2019, each year one laboratory selects non-quarantine fungal isolates from its reference collection and organizes the ILC. All laboratories identify the coded samples using their routine protocols, focusing on species-level identification and DNA extraction efficiency. Based on the results, each laboratory assesses its own proficiency in the context of its internal methods — evaluating the performance of equipment, personnel, documentation, and workflows. The process concludes with a workshop to exchange good practices and technical insights. This approach strengthens mutual understanding and collaboration.

Keywords: plant health diagnostics, DNA barcoding, interlaboratory comparisons, National Reference Laboratory.

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Advances in Pest Risk Analysis for Seed-Borne Pests: Integrating Modelling, Surveillance, and International Standards

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Seed is one of several possible pathways for the spread of plant pests, but its risk is distinct because commercial seed systems are highly regulated, have strict quality controls, and usually show a very low pest prevalence. As global seed trade increases and new diagnostic tools become available, it is important that Pest Risk Analysis (PRA) for seed-borne pests keeps pace by using modern diagnostics, modelling, and surveillance data to support fair and science-based decisions. This presentation will show how quantitative modelling, validated diagnostic data, and structured surveillance systems can make PRAs more accurate and consistent. It will also outline how international standards (ISPM 2, ISPM 11, and ISPM 38) and reference materials improve transparency and confidence in assessing seed health. For the seed sector, these advances are essential to closing the gap between what diagnostics tools can detect and how regulators use that information. Modern diagnostics tools, when coupled with modelling and surveillance data can help shape fit-for-purpose measures that take into account the biology of seed-borne pests and the high level of control in seed production systems. In the long term, integrating diagnostic, modelling, and surveillance data into PRA will support more predictive, consistent, and trade-friendly decisions, protecting plant health while enabling innovation and food security.

Keywords: seed-borne pests, pest risk analysis, diagnostics, modelling, surveillance.

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Session: Initiatives for improving plant health cooperation in diagnostics

Overview of Austria's Participation in the Euphresco Network — Including Focus on Diagnostic-Related Projects

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The Euphresco network, currently comprising over 75 members from more than 50 countries, plays a central role in coordinating transnational research in plant health. Austria has been actively involved since the network's early days, with contributions from the Austrian Agency for Health and Food Safety (AGES) and the Federal Research Centre for Forests (BFW), under the umbrella of Austria's network member - the Federal Ministry of Agriculture and Forestry, Climate and Environmental Protection, Regions and Water Management (BMLUK). In addition to project-level participation, AGES is involved in the Horizon Europe project EUPHRESCO III, which supports the strategic expansion and increased visibility of the network. A key outcome of Euphresco activities - alongside raising public awareness of plant health - is the joint establishment of targeted research projects. In addition to scientific results, these initiatives also strengthen international scientific cooperation. Over the past two decades, Austrian institutions have taken part in around 60 Euphresco projects. Austria's engagement in Euphresco demonstrates how coordinated international research fosters innovation in diagnostics, strengthens cooperation between laboratories, and supports sustainable plant health systems at both national and international levels. This poster provides an overview of Austria's involvement in Euphresco, with selected highlights of projects that include diagnostic components. Examples include efforts to improve molecular and morphological identification methods, enhance early detection tools, and contribute to validation studies. Selected projects with a diagnostic focus are e.g. 'ToMMV-detect', which validated molecular methods for detecting Tomato mottle mosaic virus. 'TEPHRIFADE' developed rapid tools for identifying quarantine-relevant Tephritidae. The 'ArthCollect' project provided DNA sequence data to support reliable identification of arthropods of phytosanitary relevance, enhancing molecular diagnostics for pest surveillance. From the forestry sector, BFW contributed for example to the 'Agrilus' project by developing an illustrated guide for identifying native *Agrilus* beetles - supporting morphological diagnostics and surveillance. These selected projects illustrate Austria's contribution to advancing plant health diagnostics through international collaboration.

Keywords: Euphresco, Austria, plant health, diagnostics, transnational research.

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Session: Initiatives for improving plant health cooperation in diagnostics

EPPO Activities in diagnostics of plant pests

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The European and Mediterranean Plant Protection Organization (EPPO) is an international organization responsible for promoting cooperation and harmonization in plant protection across the European and Mediterranean region. One of its primary activities is to provide guidance to member countries by establishing regional Standards for phytosanitary measures. Among these, EPPO Diagnostic Standards (series PM 7) aim to harmonize diagnostic procedures across the EPPO region. This set of Standards includes horizontal Standards which cover quality assurance and accreditation issues and guidance on how to perform specific methods, and pest-specific Diagnostic Standards (Diagnostic protocols) which provide guidance to detect and identify specific quarantine pests. The development of these standards is ensured by five group-specific EPPO Panels on Diagnostics (Bacteriology, Entomology, Nematology, Virology and Phytoplasmology, and Mycology) and a horizontal Panel on Diagnostics and Quality Assurance. These panels are composed of experts nominated by the National Plant Protection Organizations of EPPO member countries. In addition to Diagnostic Standards, EPPO has established the EPPO Database on Diagnostic Expertise (https://dc.eppo.int/), which provides an inventory of diagnostic expertise available within the EPPO region, with a focus on regulated pests. The database also includes validation data for diagnostic test and information on proficiency tests. EPPO also maintain EPPO-Q-bank (https://qbank.eppo.int/), a database gathering sequence data from properly documented specimens/strains present in collections. Other activities include the organization of events, such as workshops for Heads of Plant Pest Diagnostic Laboratories, training workshops and conferences on Diagnostics of Plant Pest. EPPO collaborates with other international organizations and networks to ensure that its activities are recognized internationally.

Keywords: EPPO, Diagnostics, Plant Pests, databases, collaboration.