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EPPO and Euphresco: how to ensure high quality, harmonised plant health diagnostics in the EPPO region

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EUROPEAN UNION & INTERNATIONAL STANDARDS

Introduction

Reliable and rapid diagnostic processes are essential to support inspection activities conducted by National Plant Protection Organisations (NPPOs) in the framework of their official mandate, and to evaluate the efficacy of measures taken. Official controls aim to prevent or reduce the risk of introducing new pests through the agri-food trade and to protect consumer interests. The reliability and consistency of these controls contribute to effective trade. In this context, validated and internationally accepted diagnostic protocols are of the utmost importance as they support the harmonisation of detection and identification procedures worldwide, and contribute to greater transparency and comparability in the diagnosis of regulated pests (Petter *et al.*, 2007; Petter *et al.*, 2008).

Since 1998, the European and Mediterranean Plant Protection Organization (EPPO) has been supporting the harmonisation of diagnostic methods for regulated pests in the EPPO region through the development of technical standards. As of September 2017, 130 diagnostic protocols have been approved by the National Plant Protection Organisations of the EPPO Member States. These protocols are developed by experts nominated by member countries. The EPPO Standards provide the guidance necessary for a pest to be detected and positively identified by an expert (*i.e.* an entomologist, mycologist, virologist, bacteriologist, nematologist, weed scientist, or molecular biologist), and cover different biological principles on which the tests used in different countries are based. For many years, the approach to diagnostic protocols has mostly been to include those tests that were already performed successfully in different laboratories, with validation data readily available from these laboratories. Since the establishment of the Euphresco network, a complementary approach has been followed: the need for the development and validation of new tests, or for the production of validation data for existing tests, is identified and addressed through research before drafting or revising a diagnostic protocol.

What is Euphresco?

In order to increase active collaboration among the organisations involved in plant health research activities at the national and regional levels, Euphresco (European Phytosanitary Research Coordination, www.euphresco.net) was established in 2006 and funded by the EU as an ERA-NET project. Euphresco has subsequently evolved into a self-sustaining international network hosted by EPPO. The benefits of this coordination are multiple (Giovani *et al.*, 2015; Giovani, 2017). By fostering collaboration at the research level, Euphresco enables researchers to work on common problems and contributes to the adoption of common standards and practices in research activities, including those with diagnostic aims. From the research area, these common approaches can spread to more applied activities, contributing to their harmonisation.

Every year, Euphresco members identify research priorities to be addressed through transnational collaboration. Many research projects have been funded with the aim of developing new tests for the detection and identification of pests, validating these tests or evaluating the proficiency of laboratories. Table 1 lists the most recent projects focussing on different diagnostic aspects.

Examples of the benefits of Euphresco projects

Close links have been established between Euphresco projects and EPPO activities in the field of diagnostic standardisation and these links are being developed further.

The Euphresco network brings together about 70 organisations, including all National Plant Protection Organisations (NPPOs) that are members of the EPPO. As such, they can participate in identifying the research priorities to be funded each year. Any questions arising from

EUROPEAN UNION & INTERNATIONAL STANDARDS

their routine activities can be addressed rapidly through international research collaborations that will provide scientific evidence to support the work of NPPOs. The outputs of Euphresco research projects provide valuable information for the development of EPPO regional Standards. Thus, the project 'Development and validation of innovative diagnostic tools for the detection of *Erwinia amylovora'*, the causal agent of fire blight, provided validation data on tests for diagnosis in symptomatic and asymptomatic plant material to be used for the revision of EPPO Standard PM 7/20. Given the emergency situation in the EPPO region linked to the outbreak of *Xylella fastidiosa*, EPPO revised Standard PM 7/24 in 2016 based on the experience gained in the region. With regard to the large number of host plants for this bacterium (359 plant species, EFSA 2016) and the complexity of *Xylella fastidiosa* infection (*e.g.* its uneven distribution within a plant and a potential absence of disease symptoms), a number of important knowledge gaps still need to be filled, such as field sampling, sample preparation with respect to different matrices, and diagnosis in insect vectors. Two Euphresco projects ('Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors' and '*Xylella fastidiosa* and its insect vectors Cicadella') were recently approved to be funded and will provide valuable knowledge to be used when revising the EPPO diagnostic protocol.

Improving communication

In the different EPPO countries, information may not always circulate effectively and reach the National Reference Laboratories and other laboratories involved in official diagnostic activities. However, both EPPO and Euphresco provide platforms for communicating up-to-date scientific and technical information within a large group of member countries. EPPO, as an intergovernmental organisation, has been very active in supporting the sharing of knowledge and expertise from different countries, through the activities of Diagnostic Panels, through the organisation of scientific and technical conferences and workshops, and through the establishment and maintenance of various databases (such as the EPPO Database on Diagnostic Expertise). By focussing on the coordination of plant health research, Euphresco facilitates communication among the various plant health stakeholders: research funders, National Plant Protection Organisations and research institutes, and brings them together to work on shared priorities. In Euphresco, organisations from more than 50 countries on five continents (Africa, the Americas, Asia, Europe and Oceania) collaborate on a voluntary basis to jointly address plant health challenges. Cooperation enables members to benefit from additional knowledge and skills not available in a given country or region, and more practically, to use infrastructures or carry out research on pests in areas where they already occur.

The European Reference Laboratories: enhancing cooperation in diagnostics in the EPPO region

The new EU Regulation (EU 2017/625) on official controls entered into force on 27 April 2017 and foresees the establishment of European Reference Laboratories (EURL) whose activities will enhance diagnostic capability and strengthen diagnostic activities in the European Union (EU, 2017). Since the late 1970s, the European Commission has gradually been establishing a network of Reference Laboratories covering various fields, such as animal health and animal welfare (*e.g.* the EURLs on foot and mouth disease, Newcastle disease or feed additives) and food safety (*e.g.* the EURLs on mycotoxins or pesticides in cereals). However, to date EURLs on plant pests have not been established. During this period, the plant health sector developed a number of activities to address its very specific needs. Expert groups are currently advising the European Commission on the best approach for the establishment of Plant Health EURLs. Research activities fall within the mandate of the EURLs but are rarely undertaken, mainly because other priorities absorb the limited funding available for the implementation of the EURLs' work programme. Research, and its coordination, is the main objective

EUROPEAN UNION & INTERNATIONAL STANDARDS

of Euphresco: research priorities are identified by NPPOs and research funders, and projects are funded to support their policy and provide evidence on specific questions. Collaboration with the European Commission could enable the specific needs of the EU, including those of the EURLs, to be addressed by transnational research consortia. EPPO and Euphresco are looking forward to cooperating with the EURLs as one measure to more efficiently address the everyday threats linked to regulated plant pests, and contribute to high quality, harmonised plant health diagnostics in the EPPO region.

TABLE 1/ List of the most recent Euphresco projects with a diagnostic objective (projects funded since 2015).

Title	Activity
The biology and epidemiology of '*Candidatus Liberibacter solanacearum*' and potato phytoplasmas and their contribution to risk management in potato and other crops	TD, TPS
Identification and early detection of *Cryphonectria parasitica* and *Ceratocystis platani* occurring on trees in Europe	TPS
VirusCollect II: building an international network of reference collections for regulated and other important plant viruses and viroids	RM
Assessment of *Dickeya* and *Pectobacterium* spp. on vegetables and ornamentals	TD
Consensus detection and identification protocol for *Acidovorax citrulli* on cucurbit seeds	TPS
Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors	TD, TPS, PT
Determine different *Plum pox virus* strains in wild hosts and in stone fruit cultivars with different susceptibility as a part of improved control and surveillance strategies	RM
Ringtest for improved *Potato virus Y* strain detection	PT
Tracking vectors of bacteria and phytoplasmas threatening Europe's major crops	TD
The application of Next-Generation Sequencing technology for the detection and diagnosis of non-culturable organisms: viruses and viroids	TD, TPS
Development, validation and verification of a diagnostic tool for detection and identification of *Ralstonia solanacearum* and *Clavibacter michiganensis* subspecies *sepedonicus* directly on plant tissue	TD, TPS, RM
Identification protocols for analysis of aquatic plants imports	TD
Inventory of living collections of cyst and root knot nematodes in Europe and their maintenance techniques	RM
Monitoring, detection and identification of the Japanese flower thrips *Thrips setosus*	TD
Understanding Little Cherry Viruses through improved diagnostics and insight in the occurrence and epidemiology	TPS, RM
Global warming and distribution of root-knot nematode species of the tropical group	TPS
Test performance studies on detection tests for '*Candidatus Liberibacter solanacearum*'	TPS
Development of detection methods for viruses on potato (PVT, APMoV) and APLV/APMMV test performance study	TPS
Study on the diversity of phytoplasmas detected in European forests	TPS
Assessment of a generic method for the detection of Begomoviruses	TPS
Improvement in diagnostic tests for quarantine pathogens by digital PCR	TPS, RM
Use of barcoding, from theory to practice	PT
Epitrix II	TPS
Xylella fastidiosa and its insect vectors *Cicadella*	TD, TPS
Comparison of real-time PCR detection methods for the plant pathogen '*Candidatus Liberibacter*' spp. causing Huanglongbing disease on *Citrus* spp.	TPS

TD: test development/optimisation; TPS: test performance study; PT: proficiency test; RM: reference material.

EUROPEAN UNION & INTERNATIONAL STANDARDS

More information on projects can be found here.

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