EPPO programme on diagnostics

Serving the needs of EPPO plant pest diagnostic laboratories

Scientific Session of the Commission on Phytosanitary Measures

EPPO Secretariat

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Plant Quarantine: EPPO's missions

Prevent entry and spread of pests (crops, forests, natural environments)

- Identify potential risks: Early warning systems to identify emerging risks
- Evaluate potential risks: Pest Risk Analysis
- Recommendations on pests which should be regulated as quarantine pests (EPPO A1 and A2 Lists)
- **Preparation of Standards (e.g. official control Standards, diagnostic protocols, inspection procedures….)**

Provide information to EPPO members

Why an EPPO programme on Diagnostic Protocols

Laboratory analysis are needed in the framework of inspection programmes to detect and identify pests

on consignments (imported or exported)

for the surveillance of their territory (in fields, nurseries glasshouses….), in the framework of eradication programmes

Programme started in 1998

Need for a harmonized approach to diagnostic methods for regulated pests in the EPPO region

objectives of diagnostic protocols

- to ensure correct **detection** and **identification** of regulated pests
- to ensure **harmonized** methodology in different diagnostic services/laboratories
- *More recently:* to provide **performance criteria** for tests to be recognized as reference tests under accreditation

The Panels on diagnostics
6 Panels involved in the diagnostic work

Panels	Nb of experts participating
Horizontal Panel:	
Diagnostics and Quality Assurance	20
Specialized Panels:	
Diagnostics in Bacteriology	25
Diagnostics in Entomology	15
Diagnostics in Mycology NEW	15
Diagnostics in Nematology	16
Diagnostics in Virology and Phytoplasmology	19

Terms of reference adopted for each Panel

The Panels on diagnostics: the experts

- Nominated by their NPPO, nomination endorsed by the EPPO Executive Committee.
- Members mostly from NPPOs but can come from research institutes and universities as well.

The Panels on diagnostics: meetings

- Annual meeting for the Panel on Diagnostics and Quality Assurance
- Every 18 months for specialized Panels.

Panels meet alternatively in the EPPO HQ and in member countries

Preparation of diagnostic protocols: how are priorities selected?

Pest specific DPs

- Pests from A1 and A2 list or Alert List
- Proposals made by the specialized Panels
- Priorities set by the Panel
- No specifications needed (instructions to authors exist)

Horizontal standards

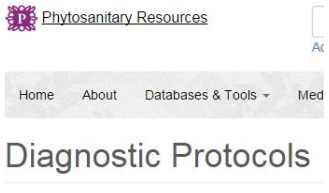
- Proposals made by the horizontal or specialized Panels
- More recently specifications presented for approval to the WPPR

Preparation of EPPO diagnostic protocols

First drafts prepared by an expert according to a common format.

- Instructions are provided to authors
- Instructions broadly in line with IPPC with some slight differences (e.g. specific instructions for the description of molecular tests):
  - To ensure that Diagnostic Protocols give the requisite information for reliable reproduction of the polymerase chain reaction (PCR) step in molecular analyses.
  - To avoid mistakes in the reaction mix composition.
  - To ensure that appropriate controls are included.
  - To harmonize the interpretation of the test results.

- Look at existing protocols e.g.



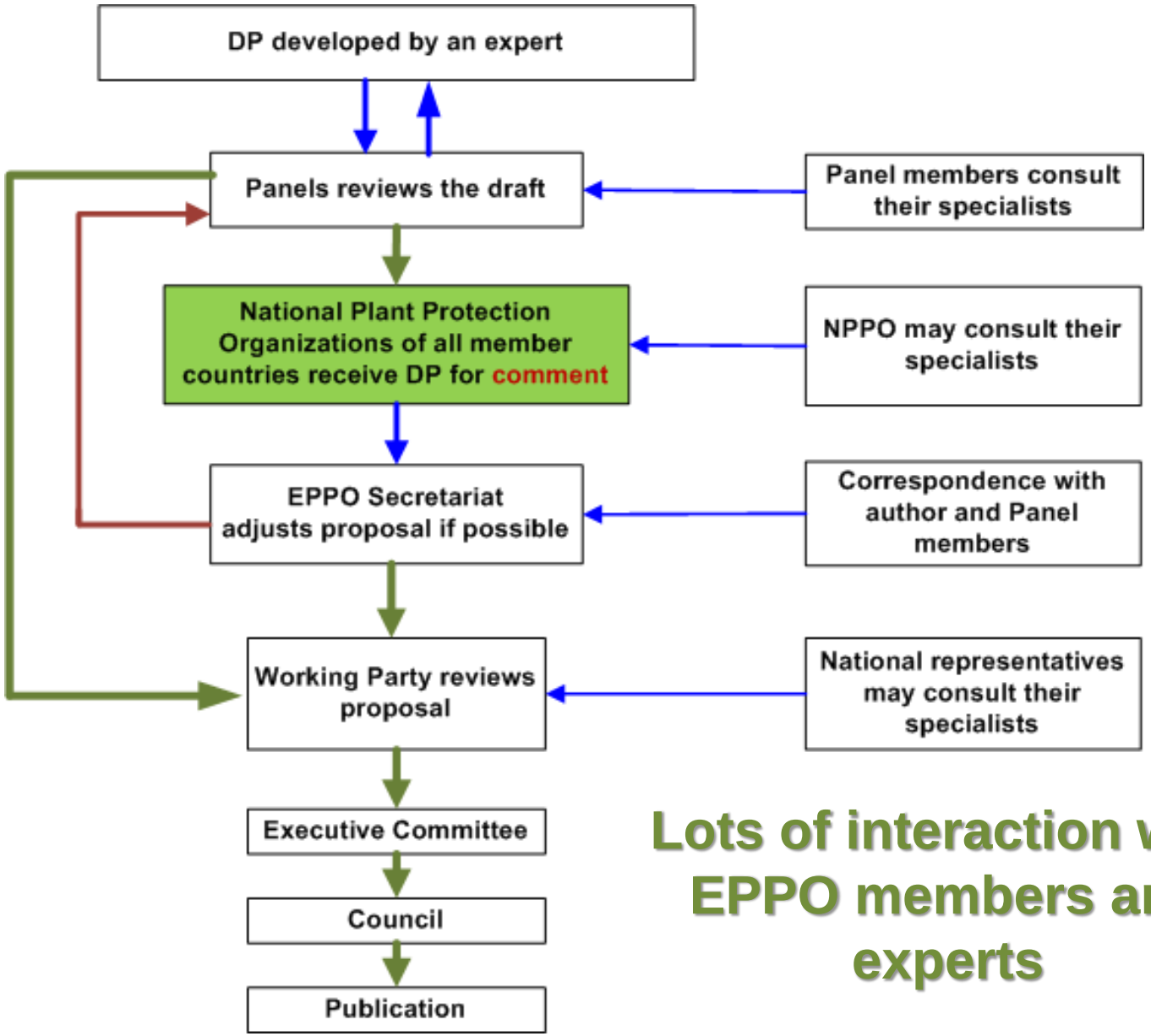
Revision of approved diagnostic protocols

- **Horizontal standards**: need for revision evaluated on an annual basis (feedback from specialized Panels possible).
  - Surveys may be organized e.g. revision of PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* was based on the results of the survey on its use in laboratories in the EPPO region.
- **Pest-specific diagnostic protocols**:
  - Volunteers selected among Panel members for each adopted protocol
  - Task
    - prepare a report to be presented at the Panel on the need for revision based on a survey of the literature
    - Identify any new test that would be appropriate for inclusion
    - Identify if tests included should no longer be recommended.

Protocols under revision are flagged on the EPPO website

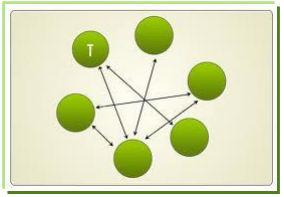
PM 7/7(1)	*Aleurocanthus spiniferus* - a new version is under preparation
PM 7/8(1)	*Aleurocanthus woglumi* - a new version is under preparation

EPPO approval procedure for DP

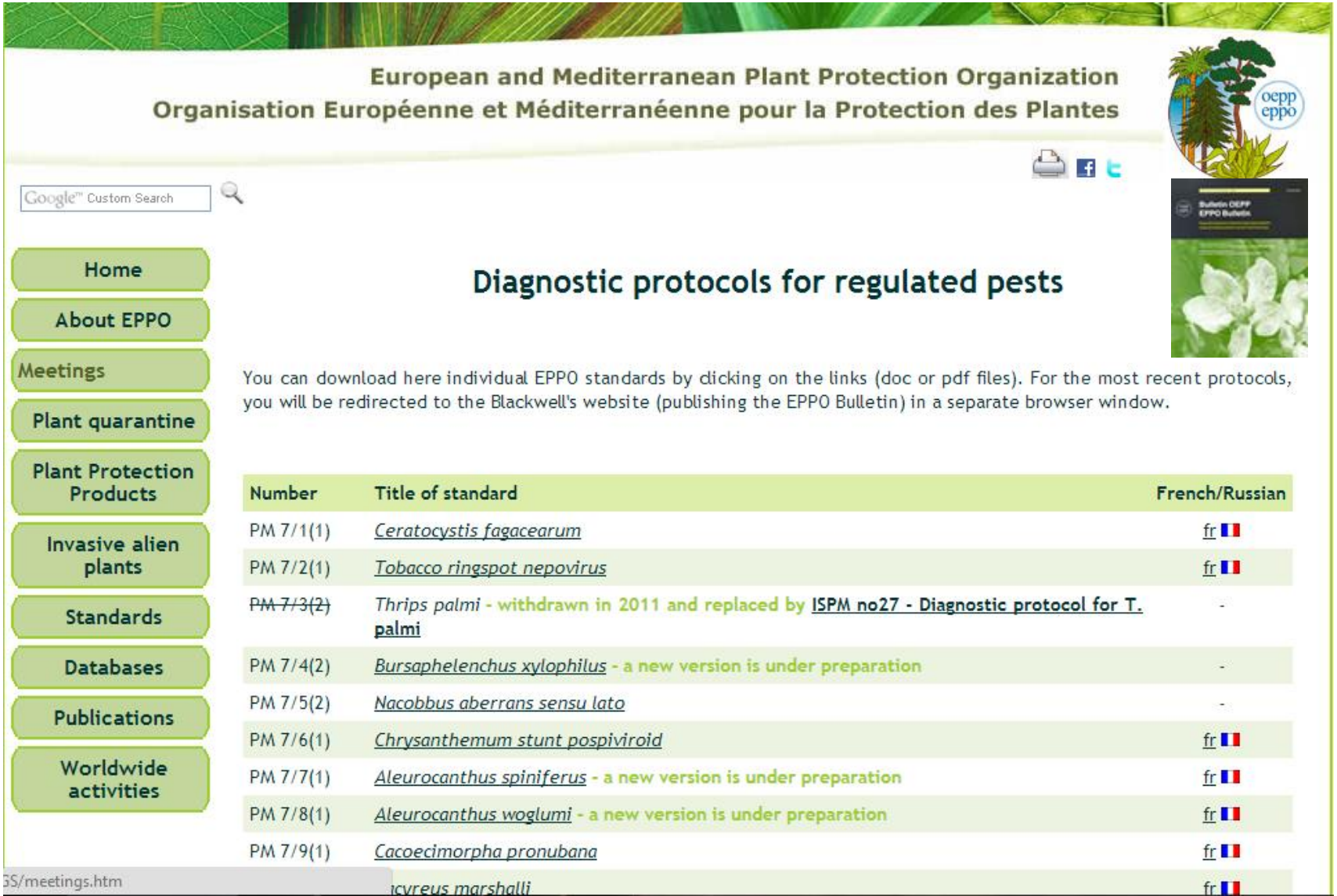


Fast track process for revisions of adopted protocols

Lots of interaction with EPPO members and experts



112 pest-specific protocols have been approved and 8 general Standards www.eppo.int



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Organisation Européenne et Méditerranéenne pour la Protection des Plantes

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Diagnostic protocols for regulated pests

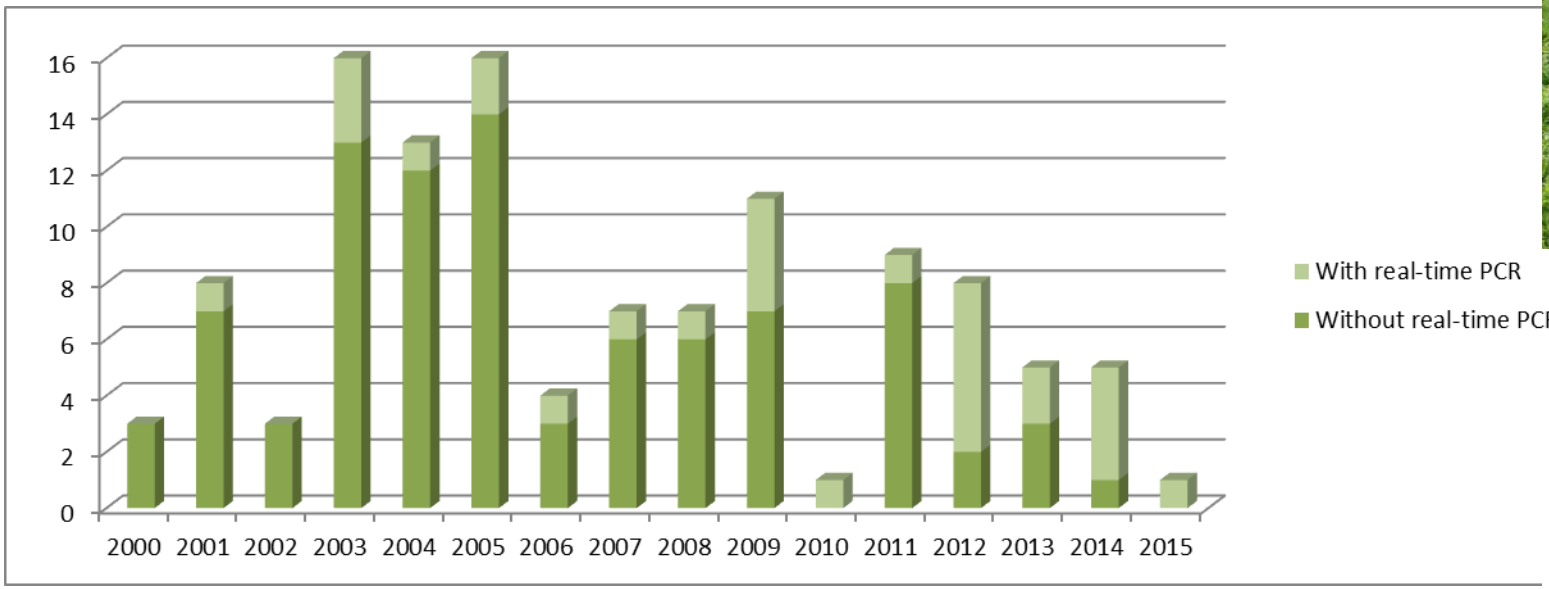
You can download here individual EPPO standards by clicking on the links (doc or pdf files). For the most recent protocols, you will be redirected to the Blackwell's website (publishing the EPPO Bulletin) in a separate browser window.

Number	Title of standard	French/Russian
PM 7/1(1)	*Ceratocystis fagacearum*	fr
PM 7/2(1)	*Tobacco ringspot nepovirus*	fr
~~PM 7/3(2)~~	*Thrips palmi* - withdrawn in 2011 and replaced by ISPM no27 - Diagnostic protocol for T. palmi	-
PM 7/4(2)	*Bursaphelenchus xylophilus* - a new version is under preparation	-
PM 7/5(2)	*Nacobbus aberrans sensu lato*	-
PM 7/6(1)	*Chrysanthemum stunt pospiviroid*	fr
PM 7/7(1)	*Aleurocanthus spiniferus* - a new version is under preparation	fr
PM 7/8(1)	*Aleurocanthus woglumi* - a new version is under preparation	fr
PM 7/9(1)	*Cacoecimorpha pronubana*	fr
	icyreus marshalli	fr

GS/meetings.htm

EPPO Protocols

- More and more real-time PCR tests but also « traditional methods »



- EPPO Standard on DNA barcoding in preparation (outcome of the EU funded project Qbol).

- Database with sequences obtained from reference materail established

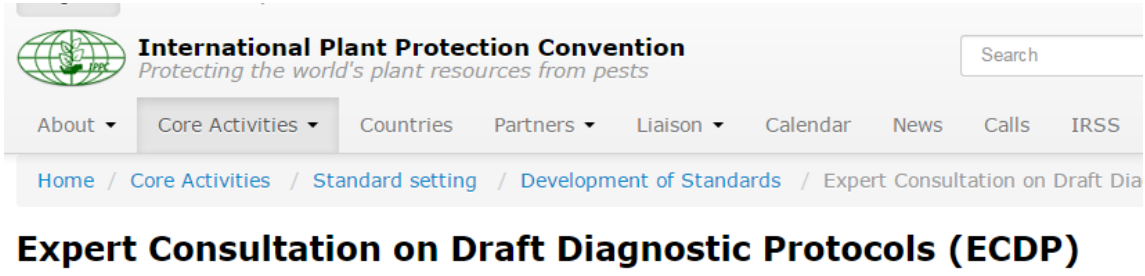
Collaboration with IPPC

EPPO and the IPPC programme on diagnostic protocols

Encouraging involvement of EPPO experts

- Nomination of experts for the TPDP (since the creation of the TPDP 3 EPPO experts have participated as members)
- Nomination of experts for drafting teams (experts from the EPPO region nominated for nearly all protocols)

- Encouraging comments from EPPO experts during the expert consultation phase.



- Coordination and preparation of regional positions on protocols sent for Member Consultation (EPPO Specialized Panels involved in the preparation of regional comments)

TPDP meetings in EPPO countries in 2004 (York), 2006 (Valencia) 2008 (Braunschweig) from 2012 to 2014 (Paris EPPO headquarters)

What happens to an EPPO protocol when an IPPC Protocol is adopted?

- EPPO protocol is revised and aligned to the IPPC one but reformatted to the EPPO format

Bulletin OEPP/EPPO Bulletin (2013) **43** (3), 431–448 ISSN 0250-8052. DOI: 10.1111/epp.12080

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/13 (2)

Diagnostics
Diagnostic

PM 7/13 (2) *Trogoderma granarium*

Specific scope

This standard describes a diagnostic protocol for *Trogoderma granarium*[1].

Specific approval and amendment

First approved in Approved in 2001–09.

Revised in 2013–09. This revision was prepared on the basis of the IPPC Diagnostic Protocol adopted in 2012 (Appendix 3 to ISPM 27). Although this EPPO Diagnostic Standard differs in terms of format it is fully consistent with the content of the IPPC Standard.

Activities on Quality assurance and accreditation

Quality assurance & accreditation

NEED FOR HARMONIZATION FOR INTERPRETATION OF ISO/IEC 17025 FOR PLANT PEST DIAGNOSTIC LABORATORIES

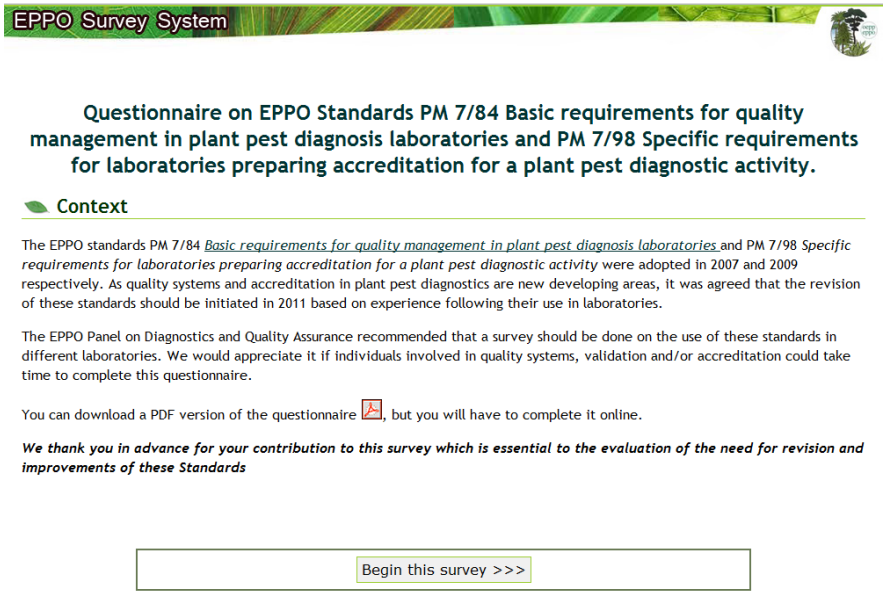
2007

Standard on basic requirements for quality assurance PM 7/84

2009

Standard on the interpretation of ISO 17025 for plant pest diagnosis (including guidance per discipline on how to validate tests) PM 7/98

Online questionnaire on the use of these standards in 2012
Revision approvded in 2014



"PM 7/98 Specific requirements for laboratories preparing for accreditation for plant pest diagnosis activities"

A laboratory preparing for **accreditation** should only use **validated tests**

Validated test = test with the following performance criteria

analytical sensitivity

analytical specificity

reproducibility

repeatability

Depending on the scope of the test **selectivity** may also need to be determined.

Validated tests providing performance criteria are considered as "standard tests" (equivalent to "standard methods" in ISO 17025).

"PM 7/98 Specific requirements for laboratories preparing for accreditation for plant pest diagnosis activities"

Laboratory performing a test

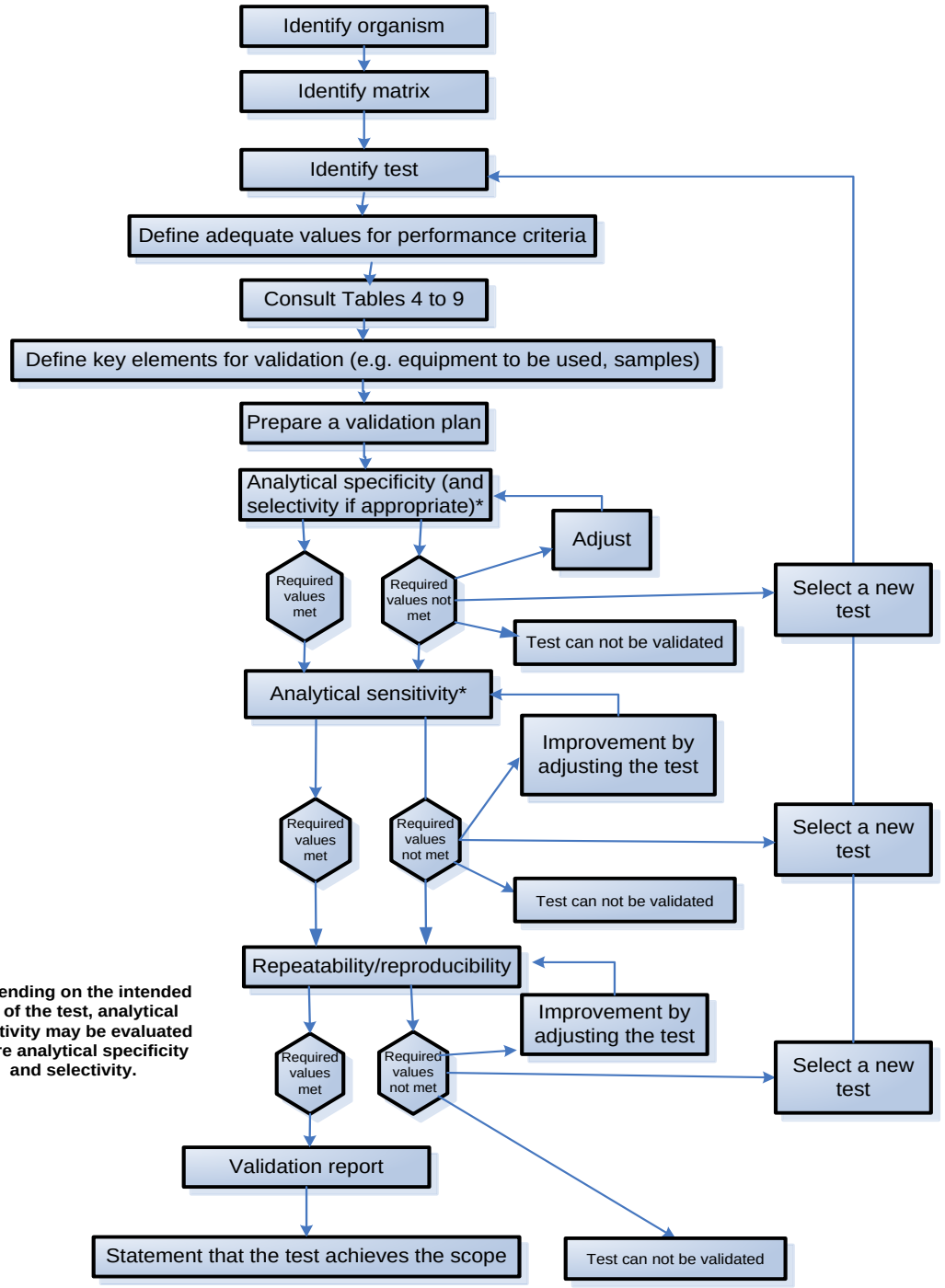
Test with validation data

Laboratory performs a verification (to confirm its competence in performing the test)

Test with no or incomplete validation data

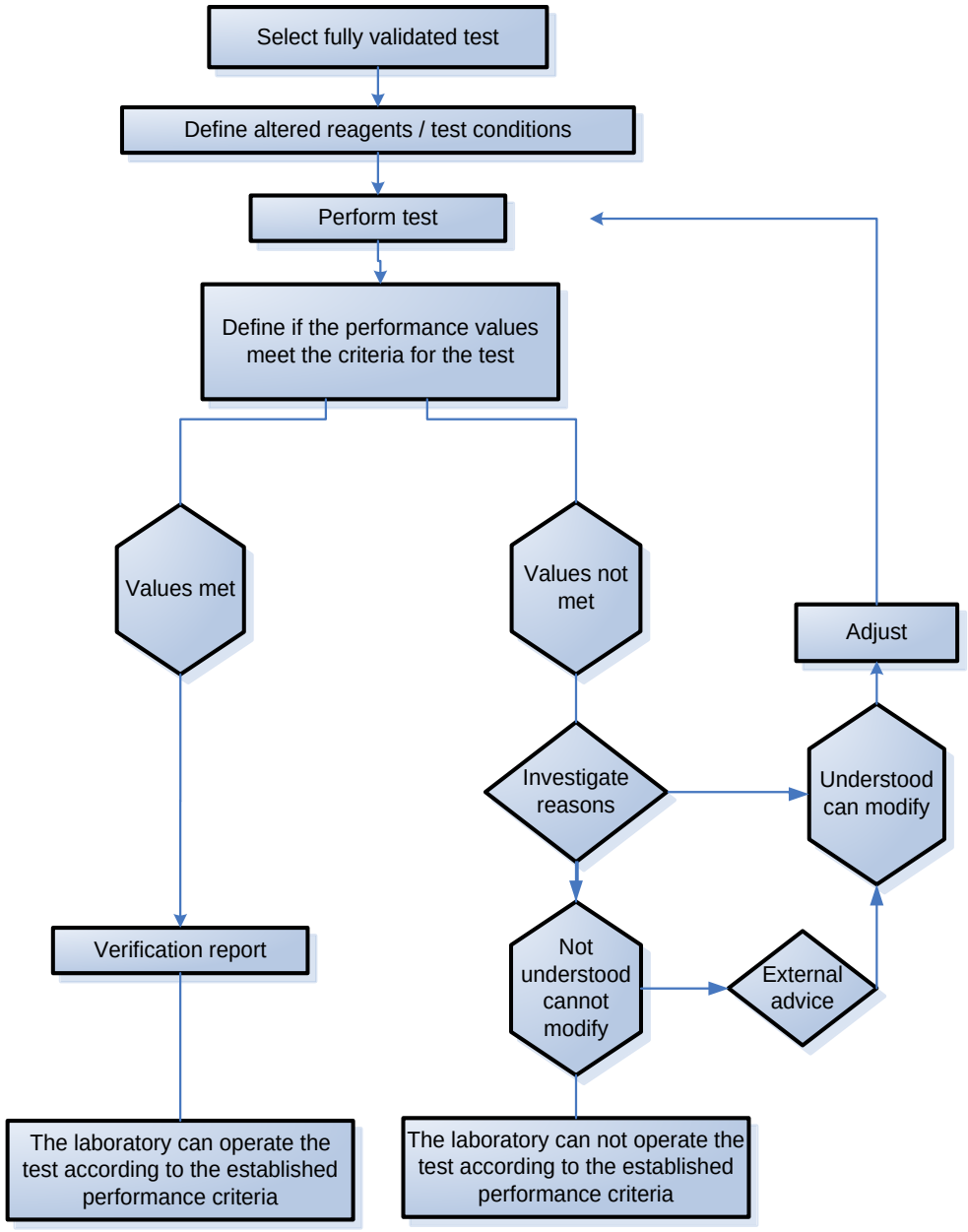
Laboratory should produce missing validation data

Validation procedure



*** depending on the intended use of the test, analytical sensitivity may be evaluated before analytical specificity and selectivity.**

Verification procedure



Validation tables bacteria (revised PM 7/98)

Molecular methods, e.g. PCR , real time PCR, LAMP Remark. This step also includes methods for isolation of DNA from the sample material.	
Analytical sensitivity	Analyse at least 3 series of spiked sample extracts with a range of 10^1 – 10^6 cells of the target organism/mL. Preferentially this is done by making decimal diluted cell suspensions of the target bacterium in the sample extracts. Determine the lowest cell density giving a positive test result. If consistent results are not obtained after 3 series, then additional series should be prepared and tested. Analytical sensitivity refers to a specific set of test parameters which should be stringently defined and standardised, e.g. brand of PCR reagents (in particular DNA polymerase) and PCR cycle conditions.
Analytical specificity	Analyse (i) strains of the target bacterium covering genetic diversity, different geographic origin and hosts and (ii) a set of non-target bacteria, in particular those associated with the sample material. Use cell suspensions of pure cultures at approximately 10^6 cells/mL. In addition, the test results can be supported by 'in silico' comparison of probe/primer sequences to sequences in genomic libraries.
Selectivity	Determine the relative insensitivity of the test to variations of the sample material, e.g. by using different hosts of the same family, different cultivars of the host plant.
Repeatability	Analyse at least 3 replicates of spiked sample extracts with a low concentration. If consistent results are not obtained additional replicates should be prepared and tested.
Reproducibility	As for repeatability but with different operator(s) if possible, on different days and with different equipment when relevant.

What about tests included in EPPO diagnostic protocols?

Not all tests include validation data

Two surveys conducted based on the use of tests included in over 60 EPPO Diagnostic protocols used in 2007 and 2013 in EPPO countries showed that many tests are widely used.

these tests are considered as giving appropriate confidence regarding repeatability and reproducibility.

+

validation data regarding analytical sensitivity and specificity to be produced only

THE LIST IS INCLUDED IN THE REVISED STANDARD

PM 7/122 (1) Guidelines for the organization of interlaboratory comparisons by plant pest diagnostic laboratories

Quality assurance & accreditation: European cooperation for Accreditation (EA) & EPPO

- Collaboration since 2007
- Joint communication EA EPPO 2009-02-23
- "recognized stakeholder" status by EA since 2011-01
- **Discussion initiated on the approach for flexible scope in plant pest diagnostic laboratories (document prepared in 2014)**

European co-operation for Accreditation

EA and EPPO agree to closer cooperation for accreditation of plant pest diagnostic laboratories

The development of robust quality management systems and the provision for accreditation are becoming increasingly important for laboratories around the world. Laboratories performing official diagnostics for regulated pests (insects, diseases etc.) need to achieve high quality standards because of the impact of their diagnosis on international trade, on the agricultural sector, and on the environment. Many laboratories within EPPO are already accredited and there is an increase in applications for accreditation among plant pest diagnostic laboratories. Accreditation of plant pest diagnostic laboratories is based on ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories*. Accreditation is given by national accreditation bodies.

One of the principal aims of the European Co-operation for Accreditation (EA)[1] consists of defining, harmonizing and building consistency in accreditation as a service in Europe by ensuring common interpretation of the standards used by its members notably ISO/IEC 17025.

The European and Mediterranean Plant Protection Organization (EPPO) [2] is an intergovernmental organization responsible for European cooperation in plant protection in the European and Mediterranean region. Under the International Plant Protection Convention (IPPC), EPPO is the regional plant protection organization (RPPO) for Europe. One of EPPO's fundamental roles is to encourage harmonization in all areas of official plant protection action. In this framework, since 1998, EPPO has established a work programme in the area of diagnostics which aims to harmonize procedures across the region by development of standards. These standards are developed by involving many national experts and they are approved through a rigorous review and consultation process. The first programme to be initiated was the preparation of diagnostic protocols for regulated pests. In addition a programme on quality assurance and accreditation has started. A standard focussing on accreditation requirements for plant pest diagnostic laboratories is being prepared and is based on the ISO/IEC 17025 Standard.

Cooperation between EA and EPPO started in September 2007 on the question of accreditation of plant pest diagnostic laboratories. EA experts attended EPPO meetings (Workshop on Quality Assurance in December 2007, the Panel on Technical requirements of diagnostic laboratories, in January 2007 and July 2008) and helped develop a scheme for accreditation of plant pest diagnostic laboratories. EPPO experts have since participated in the EA laboratory Committee meetings.

As a result of this collaboration, at the last EA Laboratory Committee meeting, it was agreed that:

- *EA and EPPO will further collaborate in order to promote and harmonize implementation of ISO/IEC 17025 in plant pest diagnostic laboratories.*
- *EA will recommend that assessors from Accreditation Bodies take note of EPPO documents when evaluating plant pest diagnostic laboratories, EPPO being a reputable technical organization working for the development of tests to be used in plant pest diagnostics*
- *EA will recommend that the EPPO expertise network will be used by accreditation bodies to achieve their missions.*

The parties have further agreed to evaluate the effectiveness of the cooperation between the parties by end 2010 with the purpose of considering options for further strengthening and formalising the relations between the two parties.

23 February 2009

[1] The European Co-operation for Accreditation (EA) is the European network of nationally recognised accreditation bodies located in the European geographical area (www.european–accreditation.org)

[2] The European and Mediterranean Plant Protection Organization (EPPO) (www.eppo.org)

EPPO collaboration in European research projects

EU Projects related to diagnostics

TESTA (2012-2015)
Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health, testing methods.

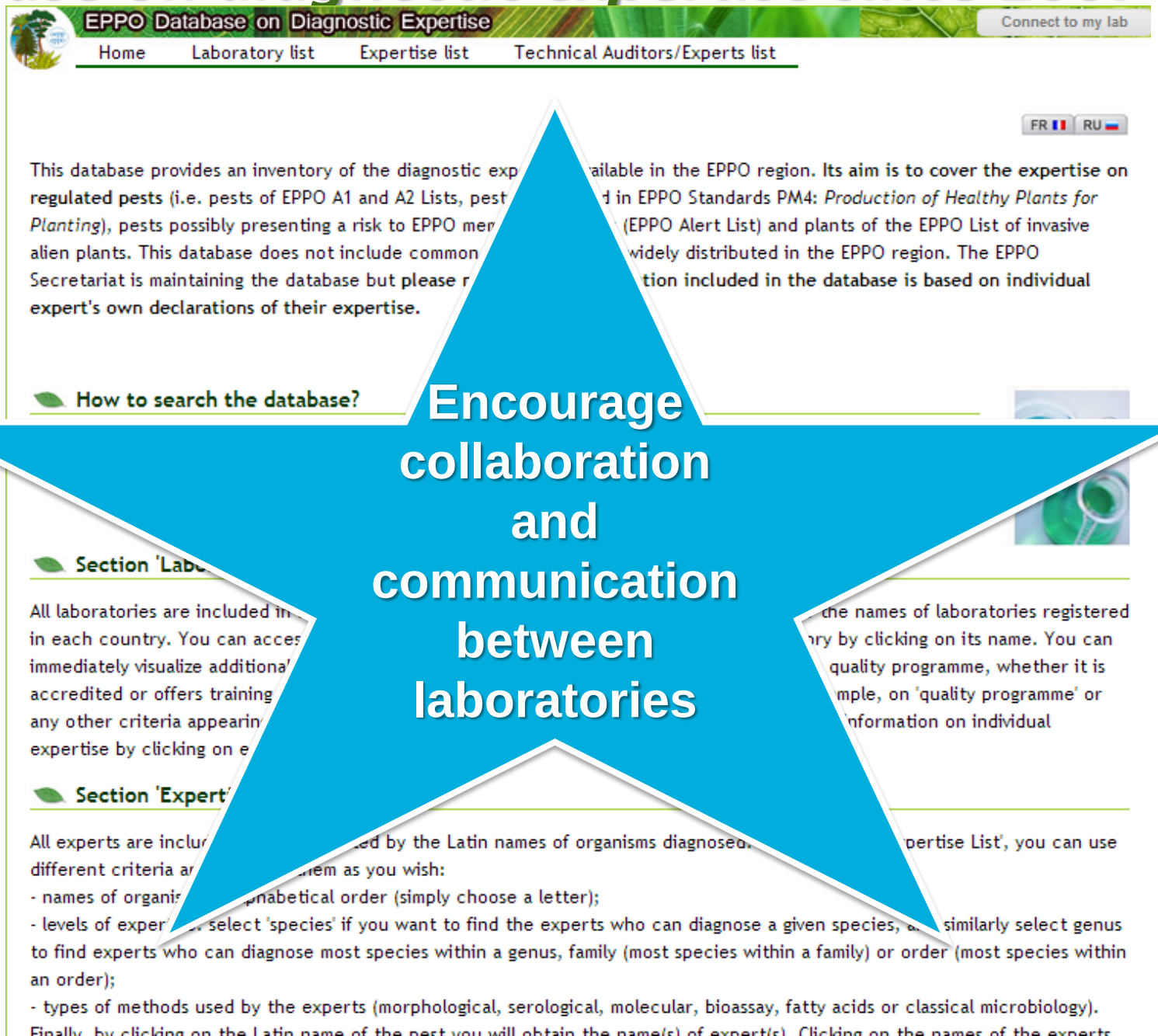
Q-collect (2013-2015)
Improved coordination and collaboration for EU Plant Health reference collections

European research coordination: EUPHRESCO

We have established diagnostic protocols and validation data are required but how could we help more….

We will build a database where experts will be able to declare their expertise …..
It should also be possible to share validation data on different tests

Database on diagnostic expertise since 2007



Encourage collaboration and communication between laboratories

http://dc.eppo.int/expertlist.php

EPPO Database on Diagnostic Expertise

Connect to my lab

Home | Laboratory list | Expertise list | Technical Auditors/Experts list | Validation data for diagnostic tests

Expertise List

Please note that the pest will only appear under its preferred scientific name given in EPPT.

* = No selection ✔ = YES ✘ = NO = Explanatory notes

Export current table

Name	Morphological	Serological	Molecular	Bioassay	Biochemical	Fatty acid profilling	Microbiology
B	*	*	*	*	*	*	*
278 records found							
Bactrocera							
Bactrocera cucurbitae							
Bactrocera dorsalis							
Bactrocera invadens							
Bactrocera latifrons							
Bactrocera tryoni							
Bactrocera zonata							
LETHMAYER Christa (Austria)	✔	✘	✘	✘	✘	✘	✘
BALMES Valerie (France)	✔	✘	✘	✘	✘	✘	✘
BALMES Valerie (France) *as Genus: Bactrocera*	✔	✘	✘	✘	✘	✘	✘
GIDRON Liat (Israel) *as Family: Tephritidae*	✔	✘	✘	✘	✘	✘	✘
DIJKSTRA Eddy (Netherlands)							

How can we help more?

Validation reports available through the database on diagnostic expertise

http://dc.eppo.int/validationlist.php

EPPO Database on Diagnostic Expertise

Connect to my lab

Home | Laboratory list | Expertise list | Technical Auditors/Experts list | Validation data for diagnostic tests

List of validation data

List of validation data available for different diagnostic tests:

Select the pest or group of pests you are interested in to access a summary sheet of validation data by clicking on

Organism	Short description	Follow EPPO/IPPC diag	Methods	-	LabID
View all taxonomic groups name starting by *	-	*	*	-	-
Aphelenchoides besseyi	Diagnostic Real-time PCR assay for identification and detection of Aphelenchoides besseyi	yes	Molecular		CLEAR
Apple proliferation phytoplasma	Detection of 'Candidatus Phytoplasma mali', 'Ca. P. pyri' and 'Ca. P. prunorum' by real-time PCR	yes	Extraction Molecular		NIB-FITO
Bursaphelenchus xylophilus	Detection of Bursaphelenchus xylophilus in wood extract with real-time PCR Leal et al. 2005	yes	Molecular		LNPVnema
Bursaphelenchus xylophilus	Identification of Bursaphelenchus xylophilus by specifc PCR Castagnone et al. 2005	yes	Molecular		LNPVnema
Bursaphelenchus xylophilus	Detection of Bursaphelenchus xylophilus in wood extract with real-time PCR Cao et al. 2005	yes	Molecular		LNPVnema
Bursaphelenchus xylophilus	Duplex PCR based identification of Bursaphelenchus xylophilus Jiang et al. 2005	yes	Molecular		LNPVnema
Bursaphelenchus xylophilus	Morphological identification of Bursaphelenchus xylophilus	yes	Morphological		LNPVnema
Bursaphelenchus xylophilus	Identification of Bursaphelenchus xylophilus by nested PCR (Takeuchi et al. 2005)	yes	Molecular		LNPVnema
Bursaphelenchus xylophilus	Specific PCR Leal et al. 2005	yes	Molecular		LNPVnema

Other activities on diagnostics: Conferences & Workshops OPEN TO ALL!!!

Workshop for Heads of laboratories Hammamet, 2013-05

EPPO Workshop on Accreditation for plant pest diagnostic laboratories York, GB, 2014-02-18/20

QBOL-EPPO Conference on DNA barcoding and diagnostic methods for plant pests Haarlem, NL, 2012-05-21/25

How can we help more

Scoop.it!

Diagnostic activities for plant pests

Information on diagnostic activities on pests and diseases of plants follow the activities of EPPO Panels on diagnostics

Curated by Petter Françoise

Biology

Paste a link to create a new scoop >>

Scoop without link | Upload your own document | Get suggestions ?

Scooped by Petter Françoise

EPPO and Diagnostics for plant pests of quarantine significance

April 29, 2012 12:26 PM

'Diagnostic activities for plant pests' is maintained by the Secretariat of the European and Mediterranean Plant Protection Organization (EPPO) and its aim is to share information on diagnostics.

EPPO is an intergovernmental organization created in 1951 which currently has 50 member countries. EPPO is responsible for harmonization and cooperation among the National Plant Protection Organizations (official authorities) of its member countries. EPPO helps its members in their efforts to protect plant health in agriculture, forestry and the uncultivated environment (standard-setting activities and exchange of information).

EPPO has established a programme on diagnostics since 1992 view the approved diagnostic protocols on the EPPO official website: http://archives.eppo.int/EPPOStandards/diagnostics.htm

On this website, a page dedicated to EPPO activities on diagnostics is also available at: http://www.eppo.int/QUARANTINE/diagnostic_activities.htm

Petter Françoise's insight:
The EPPO Secretariat maintains other scoop.it magazines onPest Risk Analysis (http://www.scoop.it/t/pest-risk-analysis) Pest Alerts (http://www.scoop.it/t/pest-alerts), Invasive Alien Plants (http://www.scoop.it/t/invasive-alien-plants), Video of plant pests (http://www.scoop.it/t/pests-on-videos), Communication on pests

Scooped by Petter Françoise

Expert Consultation on Draft Diagnostic Protocols (ECDP) - International Plant Protection Convention

From www.ippc.int - Today, 4:19 PM

" This year, experts worldwide are invited to submit comments on five different draft diagnostic protocols (DPs) under the work programme of the technical panel on diagnostic protocols (TPDP). The schedule for the Expert consultation and the tentative list of the draft DPs to comment on are as following:"

Petter Françoise's insight:
If you are an expert on the pests concerned , please participate your input is needed.

Scooped by Petter Françoise

Potential of Ypt1 and ITS gene regions for the detection of Phytophthora species in a lab-on-a-chip DNA hybridization array - König - Plant Pathology - Wiley Online Library

From onlinelibrary.wiley.com - February 19, 8:04 AM

A novel DNA-chip hybridization assay that uses the ras related GTP-binding protein 1 gene (Ypt1) was developed for the identification of several devastating Phytophthora species. The hybridization

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More information on EPPO activities in diagnostics is available on
http://www.eppo.int/QUARANTINE/diagnostic_activities.htm

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All diagnostic activities at a glance!

Since 1998, EPPO has established a work programme in the area of diagnostics to harmonize procedures across the EPPO region. This programme mainly focuses on the diagnostics of pests which are regulated as quarantine pests. The different activities conducted in this framework are described below.

EPPO diagnostic protocols

The preparation of protocols involves close collaboration between different EPPO Panels involved in diagnostics. These Panels are composed of diagnostic experts nominated by the NPPOs of the EPPO member countries. The EPPO Panels involved in diagnostics are the following (click on the links to view their composition):

- Panel on Diagnostics and Quality Assurance
- Panel on Diagnostics in Bacteriology
- Panel on Diagnostics in Entomology
- Panel on Diagnostics in Nematology
- Panel on Diagnostics in Virology and Phytoplasmology
- Joint meeting with the European Mycological Network

Each draft diagnostic protocol is initially prepared by an individual expert according to a **common format** which ensures

Thanks to our Panel members

EPPO's achievements are based on contributions from and collaboration between EPPO experts

Thanks to their NPPOs for making this possible

And thank you for your attention!