From transnational research collaboration to regional Standards: the case of the EPPO Diagnostic Protocol on *Xylella fastidiosa*

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European Commission

PONTE Pest Organisms Threatening Europe

XF ACTORS

Euphresco

EPPO IN A FEW WORDS

ONE OF 9 REGIONAL PLANT PROTECTION ORGANIZATIONS RECOGNISED UNDER THE INTERNATIONAL PLANT PROTECTION CONVENTION

CREATED IN 1951

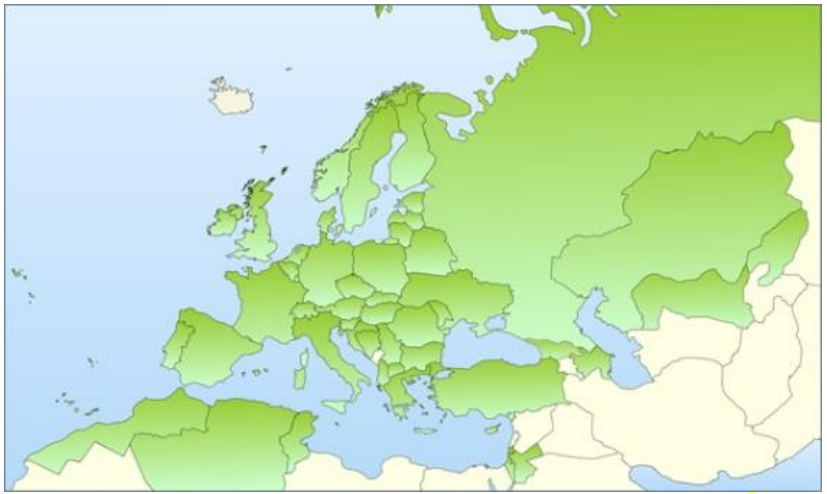
NOW 51 MEMBER COUNTRIES

Remit set out in the EPPO Convention – in practice supporting member countries in particular in the areas of:

- **Plant quarantine**
- **Efficacy of plant protection products**
- **Invasive alien plants**
- **Biological control agents**

by:

- **Developing and adopting regional technical Standards**
- **Disseminating information (information services)**
- **Facilitating networking in the region through Panel meetings, conferences, workshops.**

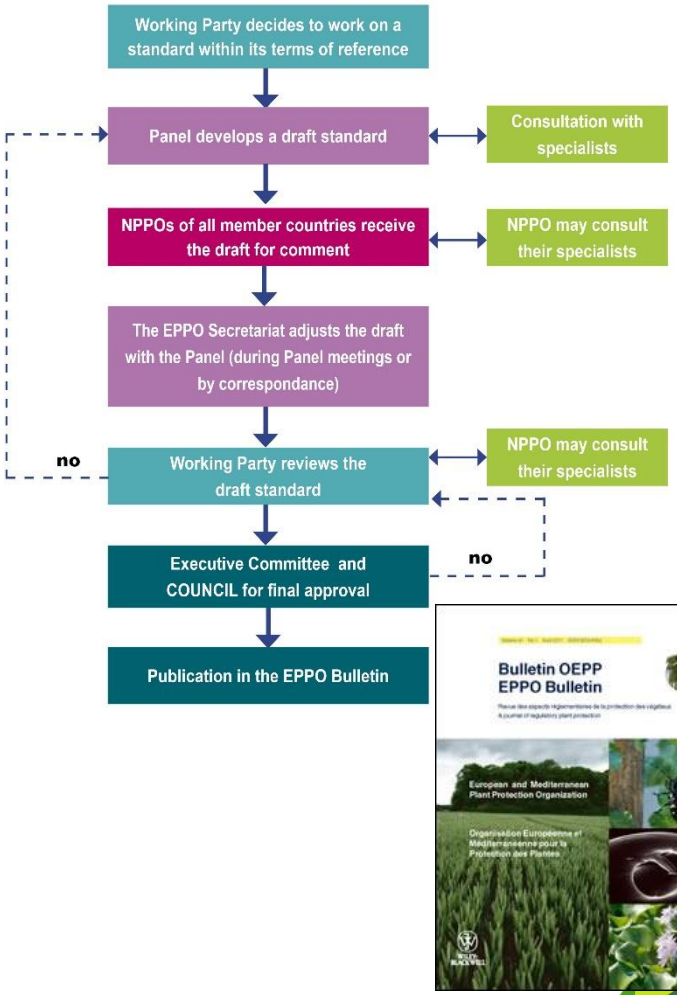


STANDARD SETTING IN EPPO

- Long standing and active program for Standard setting in several areas, including **diagnostics**.
- Objectives: to achieve a harmonized approach to detection and identification for regulated pests.
- The work started in 1998 is conducted by the Panels on Diagnostics.
- Panels are composed of specialists from EPPO member countries.

STANDARD SETTING IN EPPO

- Standards are written according to a "common format and content".
- First drafts of Standards prepared by an assigned expert author(s) or by a drafting team and reviewed by different EPPO groups of experts.
- Standards are approved following an approval procedure which involves **a formal written consultation of all EPPO Member countries**.
- Standards are published in the EPPO Bulletin and are freely available. Also available from the EPPO Global Database https://gd.eppo.int/



PM 7/24 DIAGNOSTIC PROTOCOL FOR *XYLELLA FASTIDIOSA:* HISTORY OF THE STANDARD

First version approved in 2003 (published 2004)

Focused on Vitis and Citrus

- Revision initiated in **2015** at the Panel on Diagnostics in Bacteriology
- Expert Working Group formed with experts on XF diagnostic from Austria, France, Italy, the Netherlands, Slovenia and Spain.
- Contribution received from experts from the US and Brazil

Work from December 2015 to March 2016

XYLELLA FASTIDIOSA: NOT AN EASY ONE!

Sub-species

subsp. *fastidiosa*

subsp. *pauca*

subsp. *multiplex*

+ others

vectors

More than 300 hosts

Olive

Vitis

Prunus

Polygala myrtifolia

Nerium oleander

Lavender

Coffee

.....

Validation of tests!

MAIN CHANGES: MORE PICTURES OF POSSIBLE SYMPTOMS

Fig. 3 Leaf scorch symptoms on almond. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 4 Scorch symptoms with distinct leaf burn surrounded by a dark line of demarcation between green and dead tissue. Courtesy P.M. Brennan University of Georgia (US).

Fig. 11 Symptoms of quick olive decline syndrome. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 9 Leaf scorch symptoms on *Coffea* sp. Courtesy M. Bergsma-Vlami, NPPO (NL).

Fig. 10 'Crespera' symptoms on *Coffea* sp. including curling of leaf margins, chlorosis and deformation (asymmetry). Courtesy M. Bergsma-Vlami, NPPO (NL).

Fig. 12 Symptoms of quick olive decline syndrome. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 19 Marginal leaf scorch symptoms caused by *Xylella fastidiosa* subsp. *pauca* on oleander. Courtesy D. Boscia, CNR-Institute for Sustainable Plant Protection (IT).

Fig. 20 Symptoms on *Polygala myrtifolia*. Courtesy B. Legendre, Anses, Plant Health Laboratory (FR).

MAIN CHANGES: MORE DETAILS ON SAMPLE PREPARATION

Type of sample	Host plants/ type of tissue	Minimum number of leaves per laboratory sample	Approximate weight of the laboratory sample
Samples from individual plants with leaves	Petioles and/or midribs or leaves of large size such as Coffea sp.; Ficus sp., Vitis sp., Nerium oleander.	5	0.5-1g
	Petioles and/or midribs of leaves of small size such as Polygala myrtifolia and Olea sp.	25	0.5-1 g
	Plant species without petioles or with small petiole and midrib	25	0.5-1 g
Composite sample from several plants from a single lot with leaves	Samples of asymptomatic plants collected from e.g. imported consignments or nursery monitoring	100-200	10 to 50 g
Dormant plants or cuttings	Xylem tissue	Not applicable	0.5-1 g

Asymptomatic plants tricky as often!

MAIN CHANGES: NEW TESTS FOR DETECTION AND IDENTIFICATION

Serological tests (plant material only)

- ELISA
- Immunofluorescence
- DTBIA (Direct Tissue Blot Immunoassay)

Molecular tests (plants and vectors)

- Conventional PCR (Minsavage)
- Real-time PCR (Francis; Harper)
- LAMP (Loop mediated isothermal amplification, Harper)

Different tests included to take into account the different situations in the EPPO region

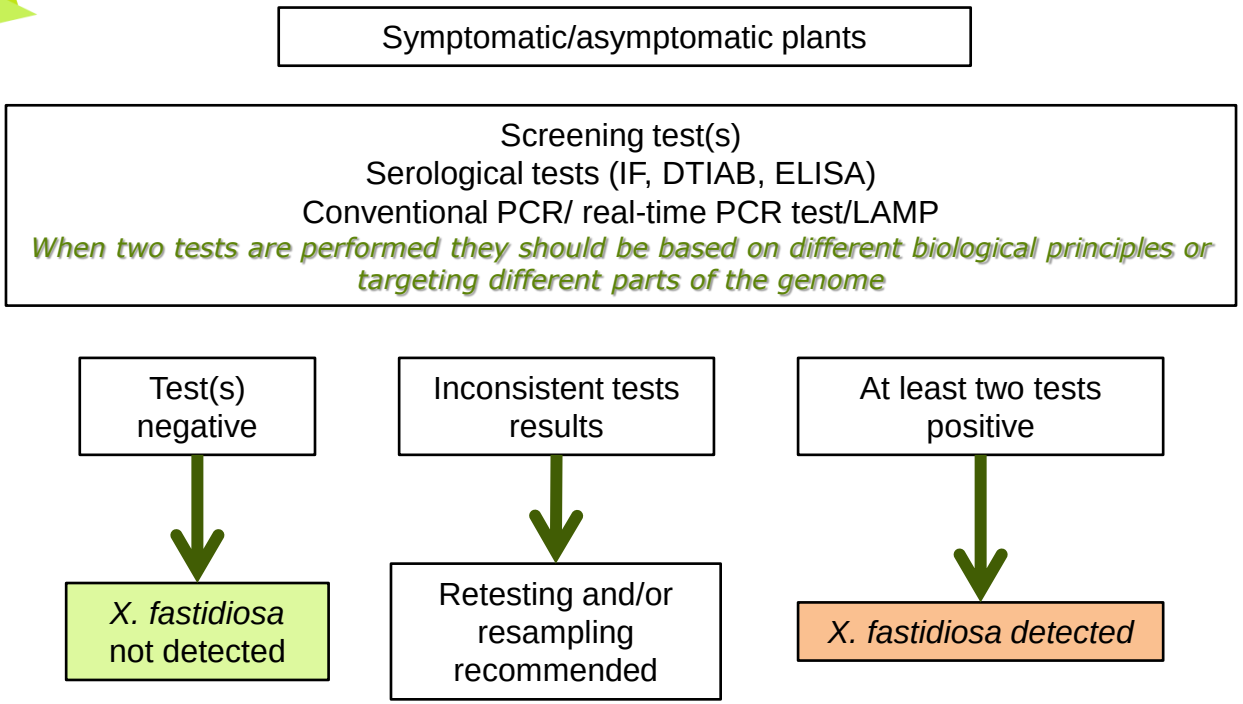
Sub species assignation:

- MLST Yuan
- Conventional PCR Pooler & Hartung ; Hernandez-Martinez (simplex & multiplex)

Unlike other protocols for bacteria isolation is not recommended as screening test.

Validation data in http://dc.eppo.int/validationlist.php

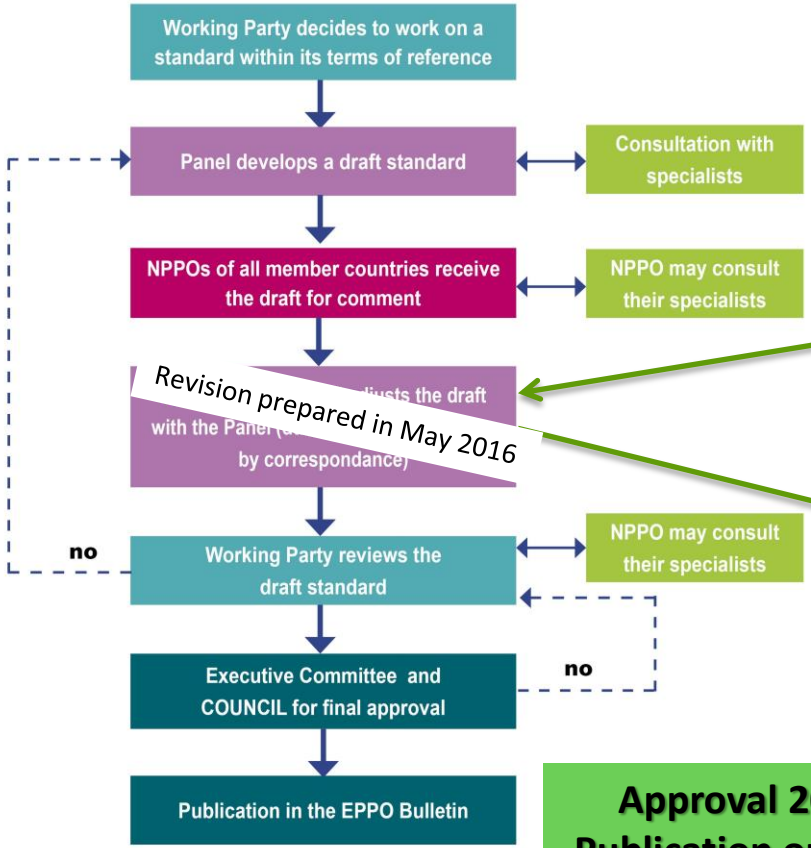
FLOW DIAGRAMS TAKING INTO ACCOUNT SPECIFIC SITUATIONS (E.G. AREA FREE OR NOT)



For testing of (a)symptomatic plants in an outbreak area or in buffer zone a single test including serological tests may be performed

It is advised to include molecular tests for detection on asymptomatic plants material in pest free area

APPROVAL IN JULY 2016



FAST TRACK PROCEDURE FOR REVISIONS OF DIAGNOSTIC PROTOCOLS

Panel prepares the revision (or EWG)

NPPOs receive the revision for comments (2016-04-03 to 2016-04-30)

Written consultation for formal objections 30 jours (2016-06-13 to 2016-07-14)

Approval if no formal objection received

Approval 2016/07/15
Publication online 2016/09


Bulletin OEPP/EPPO Bulletin (2016) **46** (3), 463-500 ISSN 0250-8052. DOI: 10.1111/epp.12327

European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes PM 7/24 (2)

Diagnostics
Diagnostic

PM 7/24 (2) ***Xylella fastidiosa***

Current version of the protocol based on the experience and validation data from the different laboratories in the EPPO region.

A LOT HAS HAPPENED SINCE MAY 2016

Several research projects on *Xylella fastidiosa* launched at national or regional level and outcomes are becoming available.

NEED TO INCORPORATE RESULTS FROM RESEARCH IN A REVISION TO ENSURE THAT THE DP IS UP TODATE

9 Feedback on this Diagnostic Protocol

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this Protocol that you wish to share please contact diagnostics@eppo.int.

NATIONAL RESEARCH PROJECTS

TRANSNATIONAL RESEARCH PROJECTS

2015-F-146: Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors (**PROMODE**)
23 research organisations (Europe, North Africa, North America)

- Develop **sampling methods** in symptomatic and asymptomatic plant materials and insect vectors
- Development of procedures for the **highly sensitive detection in host plants and vectors** (LAMP, digital PCR, NGS MLST)
- Review of available diagnostic methods and development of a common diagnostic method through **interlaboratory validation**
    - One proficiency test organized in 2017 on plant material
    - A proficiency test in progress on insect vectors

TRANSNATIONAL RESEARCH PROJECTS

Euphresco

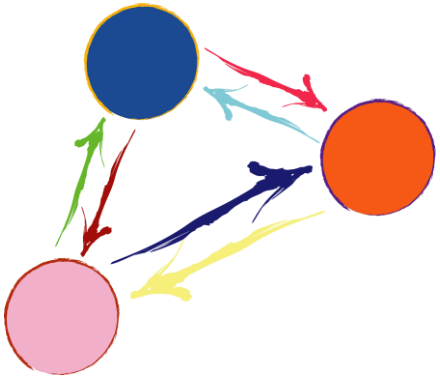
2016-F-221: *Xylella fastidiosa* and its insect vectors Cicadella
10 research organisations (Europe, North Africa)

- Evaluation of sampling methods for vector species in the environment.
- Development of real-time tests for known and suspected vector species (including North American species) to provide taxonomic support (especially nymphs) and rapid sample screening.
- Improvement of the real time LAMP detection method of *X. fastidiosa* in potential vectors.
- Validation of the non-destructive DNA extraction methods already developed in potential vectors.
- Improvement of the non-destructive extraction methods to other insect.

EU H2020 FUNDED PROJECTS

XF ACTORS

WP4 Harmonization and validation of Xf diagnostic procedures



Euphresco projects
&
national projects

POnTE
PestOrganisms ThreateningEurope

WP 4 Implementation and validation of diagnostics for early and rapid detection of target pathogens in host plants and vectors..

NEEDS FOR IMPROVEMENT OF THE EPPO DP

Revision of the DP discussed at the last meeting of the Panel on Diagnostics in Bacteriology (2017-05, Bari IT)

TPS results of projects presented

CONCLUSIONS OF THE REVIEW

The protocol broadly serves the needs of the laboratories but **most critical points** are:

- Difficulty to isolate the bacterium
- Determination of subspecies

The EPPO DP EWG should be reactivated and should include new experts from the XF-ACTORS and PONTE projects to ensure that research results are included.

EXPERT WORKING GROUP ON XF PROTOCOL

- **First meeting in September 2017 (12/13, Paris)**
  - Experts from France, Germany, Italy and Spain
- **Second meeting November 2017 (12, Mallorca)**
  - Experts from Belgium, France, Italy, the Netherlands, Spain.
  - Experts from US.

Proposals for revision made based on the data gathered by the experts in the framework of the different projects

SEPTEMBER MEETING MAIN ISSUES DISCUSSED (1)

Isolation of the bacterium

- **Addition of a sonication step before plating**

DNA extraction: more instructions for QuickPick in manual use

Real-time PCR as screening test in plant material:

Analytical sensitivity of Harper *et al.* (2010) higher than Francis *et al* (2006). The Taqman version of Francis does not detect some American strains of *Xf*

DP now recommends to perform the real-time Harper et al. (2010) first.

Real-time Li et al., 2013 under evaluation in XF-ACTORS, PONTE and Euphresco

SEPTEMBER MEETING MAIN ISSUES DISCUSSED (2)

Assignment of isolates to subspecies *(important in the European context)*

Difficulties to assign subspecies with MLST on plant extracts

some gene targets not amplified resulting in lack of amplicons to be sequenced; different host plants producing incomplete allelic profiles.

Proposal made : **sequences determined for at least two housekeeping genes can also be used for assignment of the subspecies**

Difficulty of making links between ST in MLST database and subspecies

Table correspondence between ST and subspecies added

SEPTEMBER MEETING MAIN ISSUES DISCUSSED (3)

Difficulties with MLST on different hosts or in case of mixed infections

Laboratories adapt the MLST protocol to optimise it.

Proposal made: recommendation in the DP that if erratic amplification occurs, PCR parameters can be adjusted:

- *dilution of the DNA extract (to limit inhibition) or*
- *increase of DNA input,*
- *use of a different Taq polymerase/Mastermix,*
- *decrease of annealing temperature from 65˚ C to 60˚ C or 58˚ C or*
- *increase of primer concentration from 0.3 to 0.5 µM.*

Multiplex PCR from Hernandez-Martinez *et al*. (2006)

Should not be used on plant extracts

NOVEMBER MEETING MAIN ISSUES DISCUSSED (1)

Changes proposed by the EWG accepted

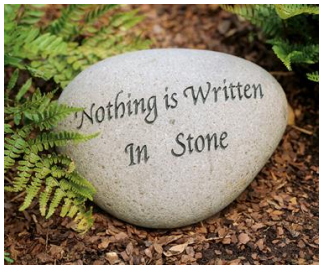
Discussion during the Euphresco meeting:

- ***Real-time PCR Harper in rare cases late Ct values observed. Comment to include in the protocol to warn the users with some recommendations.***
- ***New triplex real-time PCR developed to consider for a further revision.***
- ***Nested PCR developed***

NEXT STEPS

- **Country Consultation in early 2018 (ideally after the results of the TPS on insects and TPS on Li *et al.*, 2013)**
- **Adoption of a revision before summer 2018**
- **Planning of the next revision to start before summer 2018**

What could be improved:



Sampling of asymptomatic material

Testing of vectors

Additional validation data

New kit (AGDIA kit currently being evaluated INRA & UNIBA)

Inclusion of NGS, nested PCR…

- **Need for guidance on identification of vectors (separate protocol).**

CONCLUSIONS

- Research projects are essential for Standard setting activities in particular through:
  - the development and validation of new tests,
  - the optimization of tests or the production of additional validation data.

  But also to
  - ensure that laboratories implement the tests as expected (with e.g. organization of proficiency tests or training of laboratories)


Thank You

EPPO's achievements is only possible thanks to the collaboration of experts from our region but also from other parts of the world

Thanks to all!