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in

D'Onghia A.M. (ed.), Brunel S. (ed.), Valentini F. (ed.).
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Bari : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 121

2017

pages 19-20

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=00007200>

To cite this article / Pour citer cet article

Petter F., Baldissera G. **From science to policy, the contributions of EPPO and Euphresco to the *Xylella fastidiosa* emergency.** In : D'Onghia A.M. (ed.), Brunel S. (ed.), Valentini F. (ed.). *Xylella fastidiosa* & the Olive Quick Decline Syndrome (OQDS). A serious worldwide challenge for the safeguard of olive trees. Bari : CIHEAM, 2017. p. 19-20 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 121)



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From science to policy, the contributions of EPPO and Euphresco to the *Xylella fastidiosa* emergency

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Given the very serious threat to the agriculture and environment, *Xylella fastidiosa* was added to the EPPO A1 list of pests recommended for regulation as quarantine pests in 1981. Following the recent detection of the bacterium in Italy and France, the EPPO (European and Mediterranean Plant Protection Organization) member countries have agreed to start several activities under the coordination of the EPPO Secretariat.

In October 2013, the EPPO Secretariat prepared a specific webpage on *Xylella fastidiosa* http://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/Xylella_fastidiosa.htm which includes a brief description of the pathogen and its known vectors, the symptoms in the main host plants, its geographical distribution (with details on the most recent outbreaks in Italy and France) as well as an easy access to specific EPPO data e.g. EPPO Datasheet, EPPO Diagnostic protocols, EPPO Standards on phytosanitary procedures and other useful resources e.g. EFSA database on host plants of *Xylella fastidiosa*, EU Commission webpage on *Xylella fastidiosa*.

Besides supporting knowledge exchange in the region, the EPPO Secretariat ensures a number of other activities in plant quarantine: identification and evaluation of potential risks and development of pest risk analyses, recommendations on pests which should be regulated as quarantine pests (EPPO A1 and A2 lists), preparation of regional Standards (e.g. official control, diagnostic protocol, inspection procedures). In view of the high profile of the outbreak of *X. fastidiosa* in Europe, the EPPO Working Party on Phytosanitary Regulations agreed that the EPPO Diagnostic protocol on *X. fastidiosa* should be revised (previous version dated from 2004) and two Inspection Standards on *X. fastidiosa* should be prepared. The three Standards were prepared and sent to members for approval through an official EPPO country consultation. The National Plant Protection Organisations will provide their feedbacks on the documents whose content will be amended accordingly.

The Standards on 'Phytosanitary procedures for inspection of places of production' and on 'Phytosanitary procedures for inspection of consignments' have been prepared under the leadership of the EPPO Panel on Phytosanitary Inspections. The first document describes the procedures for inspection of places of production of plants for planting which are susceptible to *X. fastidiosa* for export or for internal country movements. The second one describes the procedures for inspection of consignments for detection of *X. fastidiosa* on host plants and insect vectors. The main content of these Standards is presented below.

Descriptions of symptoms in the main host plants are presented to support visual inspection and selection of plant material. Recommendations on how to sample are also provided. These recommendations are as follows. In the case of symptomatic plants, the sample should consist of branches/cuttings representative of the symptoms seen on the plant and containing at least 10 to 25 leaves (depending on leaf size). The Standard recommends that symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected from several plants showing similar symptoms. For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Foliage, branchlets, leaves and all accessible container surfaces, including floor or walls, should be examined to look for live insect vectors. The size of the unit of inspection (minimum number of individuals to be examined) to be selected for inspection at a specified level of infection in a specified lot size, is given according

to ISPM no. 31 'Methodologies for sampling of consignments'. To maximize the likelihood of detection, inspections and sampling during the period of active growth and after warm periods is recommended. For outdoor plants in Europe this period is usually between late spring up to autumn. For tropical plant species grown indoors such as coffee plants, sampling all year round is considered appropriate. Sampling after warm periods (e.g. late summer-early autumn) increases the probability for an accurate bacterial detection.

An Expert Working Group was formed for the revision of the EPPO Diagnostic protocol on *Xylella fastidiosa* (PM 7/24). In this Standard, recommendations for the preparation of the sample in the laboratory are provided, based on the type of sample (individual plants, composite samples, dormant plants and cuttings) and on the host plants and type of tissue (petioles, midribs, leaves, etc.). The screening tests described in the Standard are either serological (immunofluorescence, direct tissue blot immunoassay -DTBIA-, enzyme-linked immunosorbent assay -ELISA-) or molecular (conventional PCR, real-time PCR, loop mediated isothermal amplification -LAMP-). Testing for asymptomatic plants in an outbreak area or a buffer zone around an outbreak often implies that a high number of tests need to be performed. In such a situation and given that the concentration of the bacterium is expected to be higher than in an area thought to be pest free, a single test including serological tests (e.g. ELISA) may be performed. Unlike other EPPO protocols for bacteria, isolation is not recommended as a screening test as the bacterium is very difficult to isolate. Subspecies determination by molecular tests (PCR for multi locus sequence typing, conventional PCRs, multiplex PCR) and/or sequencing analysis should then be performed. Validation data for most of the tests included in the EPPO Diagnostic protocol are available from the EPPO Diagnostic Expertise Database <http://dc.eppo.int/validationlist.php>.

The EPPO Country Consultation will close on April 30, 2016. The EPPO Panel on Diagnostic in Bacteriology will meet in Paris to discuss the comments received and finalise the Standards taking into account the remarks received.

Since April 2014 the EPPO Secretariat has been hosting the Euphresco network Secretariat, thus coordinating phytosanitary research in the region. Euphresco members have funded so far ca. 60 research projects for a total budget of 12 M€, and 15 projects were funded in 2015 among which the project 'Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors (PROMODE)'. Coordinated by the CNR of Bari (Italy), the consortium is composed of 18 research institutes directly funded through Euphresco and 5 organizations that are participating with their own funds. PROMODE will provide further validation of sampling methods to effectively determine the presence of *X. fastidiosa* in symptomatic and asymptomatic plant materials and insect vectors to be used for the development of guidelines for sampling. Improved protocols for bacterial isolation from difficult matrices will be developed, and work undertaken to adapt tests (digital PCR, Next Generation based MLST) for a sensitive detection of *X. fastidiosa*. A test performance study and training workshops will be organised to support knowledge exchange and the use of best practices.

In the framework of Euphresco, an inventory of the national research projects focussing on *X. fastidiosa* was performed in 2015. Such information will allow to identify competences scattered throughout Europe, to efficiently exploit the scientific knowledge produced in order to avoid programme duplication and to ensure that valuable results are readily disseminated to the plant health community to support further research and provide scientific evidence for the policy makers.

Regular meetings are organised with other international organisations sharing an interest in *X. fastidiosa*, such as the International Plant Protection Convention Secretariat, the Near-East Plant Protection Organization, the European Commission, the European Food Safety Agency and the International Olive Council, to avoid duplication of efforts.