Sampling procedures of plant material for the survey of Xylella fastidiosa in Puglia Region, Italy

Valentini F., Cavallo G., D'Onghia A.M.

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

2017
pages 49-52

Sampling procedures of plant material for the survey of *Xylella fastidiosa* in Puglia Region, Italy

Franco Valentini, Giuseppe Cavallo, Anna Maria D'Onghia

CIHEAM, Istituto Agronomico Mediterraneo di Bari, Italy

After the identification of the bacterium *Xylella fastidiosa* in the region of Puglia, the first outbreak in Europe and in the Mediterranean basin (Saponari *et al*., 2013), and of *Philaenus spumarius* as its vector (Saponari *et al*., 2014), the National and the Regional (Puglia) Phytosanitary Service developed a surveillance plan with the scientific support of research institutions which deal with quarantine plant diseases.

The Regional Phytosanitary Service-Puglia (RPS) promptly worked out and implemented all over the region a strategic survey programme to demarcate *X. fastidiosa*-infected area, check the spread of the bacterium and set up suitable *ad hoc* measures for the control of the pathogen in compliance with the European, national and regional legislation. The work plan was elaborated on the basis of the epidemiology of the bacterium known in countries (United States and Brazil) where it was already reported on different species (grapevine, citrus) and the experience gained by scientific institutions based in Puglia in the framework of mandatory programmes to control other quarantine pathogens (e.g. *Citrus tristeza virus*, *Erwinia amylovora*). The monitoring system, type of sampling, diagnosis protocols and/or diagnostic techniques have been modified or adjusted following the scientific information acquired on the bacterium, hosts and vectors or potential vectors since its first outbreak (Loconsole *et al*., 2014; Djelouah *et al*., 2014; Elbeaino *et al*., 2014, 2014a; Saponari *et al*., 2014; Yaseen *et al*., 2015; EPPO standard PM 7/24 2). Furthermore, CIHEAM Bari experience on the use of information technology supporting monitoring enabled the development of the application XylApp (Santoro *et al*., 2014), to acquire field data (e.g. geolocalization) on the plant sample and insect as potential vector and to send data to the laboratory for analysis, and XylWeb that collect all the monitoring data (D'Onghia *et al*., 2014; Gualano *et al*., 2014).

The sampling procedures have been defined at national (Italian Ministerial Decree of 26 September) and European level (EU Implementing Decision 2015/789; Guidelines for the survey of *X. fastidiosa* in the Union territory; EPPO standard PM 7/24 based on the experience gained in Puglia to tackle the phytosanitary emergency (Regional Council Deliberation n. 1824, 5 September 2014). Since October 2013, several phytosanitary measures have been issued for the prevention and containment of the infection. They are continuously complemented, amended and updated along with the evolution of the epidemic in Italy, its outbreak in France, Germany and Spain and the new knowledge acquired by research works.

After the first infection outbreak, a wide-mesh survey was carried out all over the region using the Regional Cartographic Reticulum whose rectangular meshes, each with a surface of ca. 1,000 ha, were visually inspected and sampled if symptoms ascribable to *X. fastidiosa* were found. After the evaluation of the north limit of the infection, both the infected and buffer zone were demarcated; however, their demarcation is constantly modified after new outbreaks are reported.

Referring to the current infection status in the Italian outbreak area, a demarcated area has been defined in the EU implementing Decision 2015/789, which includes the infected and buffer zone (10km surrounding the infected zone). Surveys are conducted for early detection in the pathogen-free area. As for the demarcated area, intensive survey is conducted in the whole buffer zone and in the infected zone surrounding the buffer zone (a 20km wide strip) through visual checks and sampling, preferably from symptomatic plants. As for the rest of the area, which is considered

pathogen-free, the survey is carried out in the selected risky sites (e.g. main roads,airports, nurseries, parks, tourist areas, etc.) and in selected submeshes (about 1 Ha) intensively olive grown; the latter because olive is identified as the priority plant species for Puglia region.

In each area/zone an appropriate number of visual checks and samples are considered in relation to the risk factors which are listed in the national survey plan. In Puglia, visual checks and sampling are carried out by phytosanitary inspectors and/or agents belonging to the RPS, Forestry police or other institutions. However, personnel in charge of this activity is trained through courses provided by the RPS with the technical support of Puglia scientific Institutions actively involved in the research on this pathogen (CIHEAM Bari, CNR-Bari, University of Bari, CRSFA-Locorotondo). The field technical team is composed of at least 2 units well equipped with materials and tools for sampling (pruning shears, telescopic pruners, plastic bags, adhesive tags, cool box, etc.) and with a tablet for the use of XylApp.

Each team is tasked with the inspection and sampling of a portion of territory per day. In the buffer and containment zones, all the plants hosting *X. fastidiosa* strain CoDiRO are checked giving priority to olive, oleander and *Polygala*. The sample is taken from plants with suspect symptoms (e.g. leaf scorch, desiccated twigs/branches, etc.) or, if symptoms are absent, from a host plant selected at random in the inspected submesh.

A unit identifies the plant to be sampled (ID) and labeled the plant reporting all the data in the XylApp (georeferencing, species, varieties, symptoms, etc.), another takes the sample.

The sampling procedure on the plant, the type of sample and the sampling period depend on the host species and on the presence or absence of suspect symptoms.

- The sampling period shall coincide with that of visual checks since samples shall be taken from suspect plants. It is usually carried out from late spring to early autumn and in summer for deciduous plant species when the bacterium concentration is high. Only for olive and oleander, sampling can be made throughout the year since summer symptoms persist and the bacterium is always detectable. If symptoms are absent, it is advisable to sample in late summer early autumn when an accurate bacterial detection is higher.
- As to symptomatic plants, the plant material shall be taken from the areas close to the symptoms but not from necrotic tissues. In the event of asymptomatic plants (trees or shrubs), the sample shall be taken from the four cardinal points at different levels; it shall include non-herbaceous twig parts, and/or mature leaves with petioles from woody twigs. As regards the olive, the upper part of the canopy shall be sampled where the infection is often localized with the exclusion of young shoots, suckers or young leaves. For annual herbaceous plants, portions of stems with basal leaves shall be collected; wherever possible the whole plant with its root system.
- Each sample includes at least 8 cuttings/tree of 15-20 cm, or 10-12 mature leaves with petioles from woody twigs. It is closed in a bag and tagged with a daily identification code (ID), geographical coordinates, code of samplers, date, and presence or absence of symptoms (as generated by XylApp).
- Shake the sample before bagging it not to spread potential vectors of *X. fastidiosa.*
- Disinfect all pruning tools (sodium hypochlorite) before taking sample from a new plant.
- Complete the operations on one site, bags with individual samples shall be transferred into a larger bag; report sampling date, site and team on the tag.
- Place the samples in a cool box for transport; deliver the sample to 1st level official laboratories not later than one day after sampling, otherwise, keep the samples in a refrigerator at 4°C.

Laboratories that carry out official diagnostic assays for *X. fastidiosa* are authorized in compliance with the Italian Ministerial Decree of 14 April 1997. Before the arrival of samples, laboratories receive a list with information for each sample so as to organize assays. Upon delivery, samples are checked in an *ad hoc* premise for conformity with the indications produced by XylApp, and for the presence of anomalies (e.g. open bag, damaged sample). Information is reported in an entry register; any mistake is timely reported to SFR-Puglia. Samples eligible for testing are kept for at least 12 hours at 4°C before opening the bags to reduce the viability of potential insect vectors.

The plant tissue may be taken only and exclusively in the laboratory for the analyses of quarantine pests. The remaining plant material shall be placed in a special container for "Plant residues to be autoclaved". It is mandatory to disinfect (e.g. sodium hypochlorite solution) pruning tools for the preparation of the sample.

At the end of the above-cited operations, the plant material is closed in a bag and kept in a refrigerator for quarantine purposes till completion of diagnostic analyses. The plant material will be then autoclaved.

Figure 1. Type of sample from *X. fastidiosa* host plants: a) *Olea europaea* L. (twigs with mature leaves) and b) *Euphorbia terracina* L. (the whole plant).

References

Djelouah K., Frasheri D., Valentini F., D'Onghia A.M., Digiaro M., 2014. Setting up of Direct Tissue Blot Immuno Assay (DTBIA) for the mass detection of *Xylella fastidiosa* in olive trees. Phytopathologia Mediterranea 53 (3), 559-564.

D'Onghia A. M., Santoro F., Yaseen T., Djelouah K., Guario A., Percoco A., Caroppo T., Valentini F., 2014. An innovative monitoring model of *Xylella fastidiosa* in Puglia. Journal of Plant Pathology, 96, S4, 99.

Elbeaino T., Valentini F., Abou Kubaa R., Moubarak P., Yaseen T., Digiaro M., 2014. Multilocus sequence typing of *Xylella fastidiosa* isolated from olive affected by "Olive Quick Decline Syndrome (OQDS)" in Italy. Phytopathologia Mediterranea, 2014, 53 (3), 533-542.

Elbeaino T., Yaseen T., Valentini F., Ben Moussa I.E., Mazzoni V., D'Onghia A.M., 2014a. Identification of three potential insect vectors of *Xylella fastidiosa* in Southern Italy. Phytopathologia Mediterranea 53(2), 328-332.

EPPO Standard PM 7/24 (2) *Xylella fastidiosa*, 2016. Bulletin OEPP/EPPO 0 (0), 1–38 ISSN 0250-8052. DOI: 10.1111/epp.12327

Loconsole G., Potere O., Boscia D., Altamura G., Djelouah K., Elbeaino T., Frasheri D., Lorusso D., Palmisano F., Pollastro P., Silletti M.R, Trisciuzzi. N., Valentini F., Savino V., Saponari M., 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. Journal of Plant Pathology, 96 (1), 1-8.

Santoro F., Favia G., Valentini F., Gualano S., Guario A., Percoco A., D'Onghia A.M., 2014. Development of an information acquisition system for field monitoring of *Xylella fastidiosa*. International Symposium on the European outbreak of *Xylella fastidiosa* in olive October, 21-22 2014 Gallipoli (Lecce), Italy.

Saponari M, Boscia D., Nigro F. and Martelli G.P., 2013. Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Puglia (Southern Italy). Journal of Plant Pathology 95 (3), 668.

Saponari M., Loconsole G. Cornara D., Yokomi R. K., De Stradis A., Boscia D., Bosco D., Martelli G. P., Krugner R., Porcelli F., 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Puglia, Italy. Journal of Economic Entomology 107, 1316–1319.

Yaseen T., Drago S., Valentini F., Elbeaino F., Stampone G. and A. M. D'Onghia, 2015. On-site detection of *Xylella fastidiosa* in olive trees (*Olea europaea* L.) and insects using the real-time loop-mediated isothermal amplification method. Phytopathologia Mediterranea, 54, 3, 488−496.