CIHEAM/IAMB innovative tools for early surveillance and detection of Xylella fastidiosa

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Xylella fastidiosa is a xylem-limited, gram-negative bacterium, infecting about 380 plant species worldwide, in most of the cases without causing symptoms. Typical symptoms are leaf scorching and leaf wilting, eventually followed by the death of the plant. Any xylem sap-feeding insect is a potential vector for this pathogen. Four subspecies are officially recognized: *fastidiosa*, *pauca*, *multiplex* and *sandij*. *X. fastidiosa* appeared in the southern part of Italy in 2013, as first report in the EPPO region, causing the death of million olive trees, with severe economic, environmental and social consequences (Saponari *et al*., 2013). The strain CoDiRO (**Co**mplesso del **Di**sseccamento **R**apido dell'**O**livo) found in olive trees and several other host plant species induces the Olive Quick Decline (OQD) and is genetically identical to a strain from oleander in Costa Rica (Elbeaino *et al*., 2014). The only assessed vector is the Aphrophoridae *Philaenus spumarius* (Saponari *et al*., 2014). There is no record of successful eradication of *X. fastidiosa* once established outdoors due to its broad range of plant hosts and vectors. However, the only control means are the prevention of pathogen introduction in free areas (e.g. use of healthy propagating material) and the containment of the outbreak where the pathogen is not well established yet (e.g. eradication of infected plants and vector control).

The severe threat posed by *X. fastidiosa* in Italy prompted the Italian Ministry of Agriculture to declare a state of emergency for *X. fastidiosa*. To this aim, a special Commissioner was soon appointed and a national scientific committee was established for advising technical decisions. An action plan was established for implementing measures as for the: - removal of host plants located near roads, canals, green areas; - control of young stages of the vectors on ground vegetation; - phytosanitary treatments for the control of adult vectors; - removal of infected plants; - destruction of host species in nurseries; and other horizontal activities.

Based on the infection status, a demarcated area was established, which includes the infected zone and buffer zone (10km surrounding the infected zone). In the infected zone, where the pathogen is considered established, measures concern planting prohibition of EU host plants, while intensive monitoring and the removal of infected plants are restricted to a 20km-wide strip at the border with the buffer zone. In the buffer zone, where the pathogen is not present, measures concern the intensified monitoring of specified plants, vector control, movement restrictions out of the buffer zone. In all the above zones, the infected plants have been mapped and the management of the monitoring data has been fully computerized. The graphical representation of the areas monitored and their results are available on the official website of the Puglia Region (www.emergenzaxylella.it).

The early surveillance and detection of *X. fastidiosa* is so difficult that it is necessary to develop an efficient and sustainable management of the infection. It should be based on a thorough knowledge of the: territory (e.g. cartography, land cover), time and space evolution of the infection since its first outbreak, priority risky sites to be monitored, diagnostic protocols to be applied, etc.. The surveillance flow of both qualitative and quantitative data should be managed properly in order to provide accurate indications to the National Plant Protection Body for the application of control measures. Relatively new approaches as the remote sensing coupled with the availability of large-scale datasets, the rapid development of computer technology and biotechnology, are leading to considerable improvements in strategic and tactical decision making on plant disease

surveillance and management. To this aim, the system developed by CIHEAM Bari for the official surveillance of *X. fastidiosa* in South of Italy is aimed to early detect the pathogen integrating innovative tools for: territorial analyses (e.g. photointerpretation of aerial images), accurate on-site data acquisition (XylApp), rapid on-site pathogen detection (DTBIA, real-time LAMP) in plant material and 'spy insects' (D'Onghia *et al*., 2014; Lacirignola *et al*., 2015).

This surveillance system, which is multidisciplinary, multifunctional and multi-actors, allows the traceability of different types of data which converge in a central server (XylWeb) for their rapid storage and analysis. The main components of this system are hereafter briefly described.

The assisted photo interpretation of high resolution aerial images was developed for the rapid recognition of olive trees showing OQD-like symptoms on a large scale, being this species the primary host of the CodiRO strain in Puglia region (Gualano *et al*., 2014). This approach allows the implementation of precision intervention at local and territorial levels.

All suspected infected sites were investigated by visual observations for confirming OQD symptoms and assessing the presence of the pathogen. This method can also provide indications on: the presence of symptoms in olive trees which have been pruned before visual inspections; the application of some measures included in the action plan (e.g. soil tillage in spring to significantly reduce vector juveniles' populations), etc.

XylApp is an application for "android systems", designed and developed with the aim of facilitating, optimizing and rationalising collection, geolocalisation and storage of data related to plant (e.g. OQDS photointerpreted trees) and/or insect samples (vectors and spy insects) collected in the field during the monitoring phase (Santoro *et al*., 2014). The application consists of five independent modules for on-site data acquisition by inspectors: *Sampling, Explore & Sampling, Find, Archive* and *Vademecum.*

The spy insects approach is based on the monitoring of insect vectors or potential vectors of *X. fastidiosa*, which have been assessed to harbour the pathogen as *P. spumarius*, the only assessed vector, *Neophilaenus campestris* and *Euscelis lineolatus* (Elbeaino *et al*., 2014; Ben Moussa *et al*., 2015). The detection of the pathogen in these insect species can early reveal the presence of the infection before symptom development in the buffer zone and in the pathogen-free area. Due to the different dynamics of seasonal population of the spy insects in Puglia region, their monitoring can be carried out during the whole year.

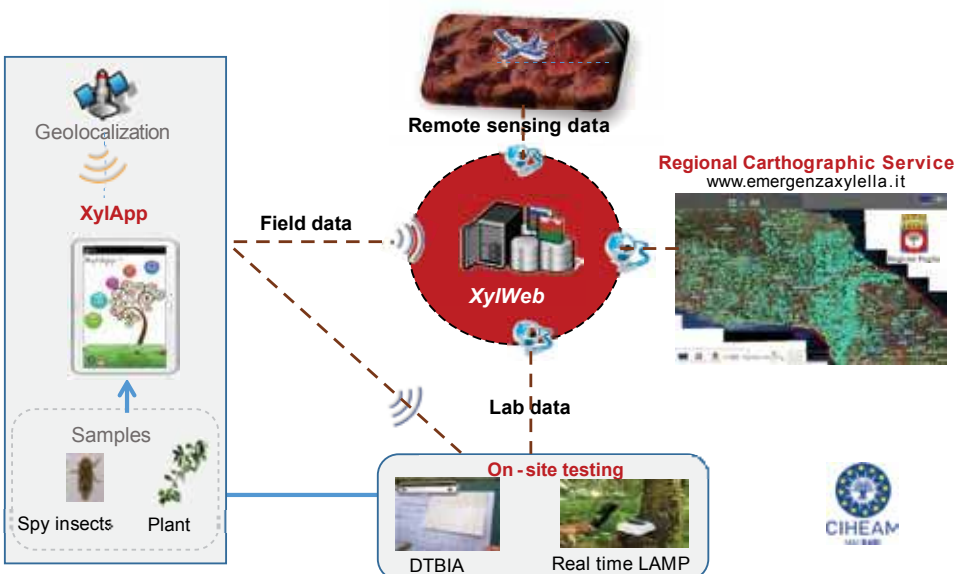
On-site rapid detection of *X. fastidiosa* has been developed using the real time LAMP (loop-mediated isothermal amplification) and DTBIA (Direct Tissue Blot ImmunoAssay). The real time LAMP can be totally performed on site in plants and 'spy insects' using a field device (Yaseen *et al*., 2015) while, in the case of DTBIA, membranes can be printed in the field with plant material and processed in laboratory (Djelouah *et al*., 2014). However, in both cases the movement of infected plant material in Xylella-free areas for pathogen testing can be avoided.

XylWeb is a web-based software for the collection, storage and management of surveillance data for *X. fastidiosa* (Gualano *et al*., 2014). This software represents the core of the surveillance system in which all data converge, e.g. daily data acquired by XylApp are transmitted in real time to XylWeb. XylWeb allows data traceability and real time analyses for producing reports and other elaborates. The application consists of the following independent modules: *Sample; Processing; Browse; Management; Downloads;* and *Links.* Its implementation with the regional cartography provides a clear map on the distribution of the samples, infected plants, etc..

In the framework of this surveillance system in South of Italy, more than 100 000 diagnostic tests have been conducted (in the buffer zone and the 20km-wide strip of the infected zone surrounding the buffer zone) with the aim of determining the presence and spread of the infection, thus applying eradication/containment measures as indicated in the Commission Implementing Decision EU 2015/789.

Thanks to the ongoing research at national and EU levels (e.g. Horizon 2020 *Xf*-Actors), the surveillance system for *X. fastidiosa* will be enhanced exploiting the potential of hyperspectral and thermal data for early pathogen detection (using manned or unmanned vehicles), smart applications (e.g. dedicated Apps for end users) and prediction models of ecophysiological stress responses of olive trees, of spatial and temporal spread of the infection, etc..

The innovative system for early surveillance of *Xylella fastidiosa*



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