Contribution of CIHEAM-Bari for the early surveillance of *Xylella fastidiosa* and its vectors on olive trees in Italy

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Introduction

The Mediterranean area is home to a wide biodiversity of plant species which must be protected for social, economic and environmental reasons. A sustainable protection strategy is therefore more essential than ever in order to protect the whole region from phytosanitary threats and genetic erosion.

For about 30 years, CIHEAM-Bari has made great efforts in the monitoring and control of harmful organisms in Italy (Apulia region) and in several Mediterranean countries, especially those of quarantine importance in order to prevent their entrance, establishment or spread (i.e. *Citrus tristeza virus, Plum pox virus, Erwinia amylovora*, etc.). To this aim, Bari Institute has promoted the use of 'healthy' propagating material, the establishment of pest surveillance programmes and application of eco-sustainable control methods. In the framework of Plant Protection Master of Science and cooperation programmes, numerous students have been trained and several research activities are carried out on phytosanitary problems affecting mainly Mediterranean fruit crops. Most of the results are transferred to the Mediterranean countries following the CIHEAM mission. CIHEAM-Bari is recognized by the Italian Ministry of Agriculture as a quarantine centre and its laboratory is accredited for the maintenance and manipulation of several pathogens.

To combat these phytosanitary threats the Institute has developed innovative solutions in early pathogen surveillance on regional scale such as efficient sampling methods, rapid diagnostic tools and predictive maps of the infection. In particular, about *Xylella fastidiosa* (*Xf*), a quarantine pest reported for the first time in Europe and in the Mediterranean area only in 2013, CIHEAM-Bari has played a pivotal role in research, training and support to the Apulian Plant Protection Service (PPS) in the bacterium monitoring.

X. fastidiosa, a gram-negative plant pathogenic bacterium is a regulated quarantine pest, whose introduction and spread into EU Member States is banned. Four subspecies of the bacterium have been described: ssp. *fastidiosa* (the agent of Pierce's disease of grapevine in California), ssp. *pauca* (the agent of Citrus variegated chlorosis in Brazil), ssp. multiplex and ssp. sandyi, each showing a high degree of host specificity.

X. fastidiosa ssp. pauca strain CoDiRO (Complex of rapid olive decline) is the bacterium which was first reported under field conditions from the province of Lecce (Apulia region, southeastern Italy), in association with a devastating disease of olive known as "olive quick decline syndrome" (OQDS). OQDS symptoms consist of leaf scorch and desiccation of scattered terminal shoots, which rapidly expand to the rest of the canopy, and result in the collapse and death of the tree. This pathogen was found to be transmitted by a xylem fluid-feeding insect vector, the polyphagous meadow spittlebug *Philaenus spumarius* (L.) (Hemiptera: Aphrophoridae), while *Neophilaenus campestris Fallén* (Aphrophoridae) and *Euscelis lineolatus Brullé* (Cicadellidae) were reported to be additional potential vectors. Apart from olive, other host species of the bacterium have been identified, most of which are ornamentals or belongs to the typical Mediterranean bush.

Upon the request of the Apulian PPS, a monitoring campaign was immediately organized in the region to assess the distribution of the pathogen and its putative vector, and to limit further dissemination of the bacterium. CIHEAM-Bari is one of the laboratories where samples are officially tested and research conducted on this newly introduced pathogen. Moreover, CIHEAM-Bari launched different research lines on *Xf* soon after its first report in 2013, mainly in the framework of the CIHEAM Master of Science programme in Integrated Pest Management, which is focused on (i) pathogen isolation and characterization; (ii) improvement of pathogen detection tools; (iii) identification of potential insect vectors/spies; (iv) evaluation of different control methods for pathogen and vector; (v) development of pathogen surveillance tools by remote sensing and information technologies. Results already achieved and presented at national and international scientific events or published in scientific journals were also put at disposal of the Apulian PPS for the implementation of *Xf* monitoring programme.

In particular, an efficient and innovative surveillance system of *Xf*, integrating advanced tools of territorial analyses and diagnosis methods of the bacterium in plant material and in the spy insects was designed and developed by CIHEAM-Bari (D'Onghia et *al.*, 2014 Fig. 1). This system, part of which is already officially applied in Apulia, is composed of a central web server (XylWeb) in which data from processed high resolution aerial images (Photointerpretation of OQDS trees), field smart application on android device (XylApp) and on-site rapid diagnostic assays (DTBIA and real time LAMP) converges (Fig. 1).

The spy insects approach (Elbeaino et *al.*, 2014)

An effective approach included in the innovative surveillance programme for *Xf* is the use of *spy insects* as bioindicators to assess the presence of the bacterium in areas which are considered free because of the absence of specific OQDS symptoms.

The *spy insect* is able to harbor the pathogen by feeding from infected plants; therefore it can be used as a sample, instead of the plant material, for testing the presence of the pathogen in a specific area. Apart from *P. spumarius*, which is also vectoring the bacterium, *N. campestris* and *E. lineolatus* proved to be *spy insects* by harboring *Xf*. This approach is very important in order to define an area as *Xf*-free, and to detect the pathogen before symptoms develop. However, it is very hard to assess the presence of the pathogen in asymptomatic trees due to the uneven distribution of the bacterium inside the host plant.

Photointerpretation of aerial images (Gualano *et al.*, 2014)

The availability of remote and proximal sensing instruments suitable for running in stand-alone or web-based GIS platforms has led CIHEAM-Bari to a significant progress in the large scale surveillance of infections related to pathogens of quarantine importance (i.e. *Citrus tristeza virus*). As for *Xf*, the photointerpretation technique was developed to recognize and classify the canopy with leaf scorching and desiccation as OQDS-suspected plants in GIS environment and used in the official large-scale monitoring of the bacterium. For this purpose, high resolution aerial images were used by processing visible (VIS) and near infrared (NIR) data for a rapid identification of appropriate photo-types, morphologically suitable for detecting the OQDS alteration. The identified OQDS- suspected trees are geo-localized for field inspections, sampling and testing. This approach is very innovative for a precise field monitoring, thus reducing time and costs. Moreover, this tool also provides a quantitative and spatial analysis of the occurrence of OQDS trees in different areas.

XylApp (Santoro *et al.*, 2014)

The smart application named XylApp has been designed for Android devices in order to optimize, rationalize and make sampling, geo-localization of the investigated sites and samples, data storing and transmission to the central server XylWeb easier. This application is composed of five independent modules: "*Sampling*" which allows to acquire sample data taken from the field without map support; "*Browse and sampling*" to acquire sample data from the field with the map support using the regional cartographic grid; "*Find*" to pinpoint one or more targets through geographic coordinates; "*Archives*" to (i) store field data in a directory, (ii) send them to a remote database, (iii) generate a report in Excel format, (iv) edit and/or delete single samples; "*Vademecum*" which provides a valuable guide with the main knowledge about host species, symptoms, insect spy/vector, equipment, etc.. In the official Apulian monitoring of *Xf*, XylApp is provided free of charge to the plant protection agents who have been trained for the use of this application in the field with a great success for the programme.

Rapid pathogen detection techniques (Djelouah *et al.*, 2014; Yaseen *et al.*, 2015)

Several detection methods, including tests such enzyme-linked immunosorbent assay (ELISA), immunofluorescence, Polymerase chain reaction (PCR)-based and Loop mediated Loop-mediated Isothermal Amplification (LAMP) assays, have been used over the years to detect *Xf* in grapevine, citrus, almond and other hosts.

Soon after the first findings of *Xf* in Apulia, no information was available on the strain or genotype infecting olive trees, nor procedures for the identification of the bacterium in locally grown olive cultivar tissues. Thus, the validation of ELISA and PCR protocols was necessary even before a large scale monitoring program for *Xf* detection in Apulia. The CIHEAM-Bari laboratory was part of a ring-test with other accredited laboratories and ELISA was identified as the official diagnostic assay in large-scale monitoring whereas PCR as a method for the confirmation of ELISA positive/doubtful samples in new infected areas.

In order to reduce time and costs for testing, and to avoid the high risk associated with the movement of the infected plant material to 'pathogen-free' areas, in which most of the laboratories are located, CIHEAM-Bari has developed the protocols of two diagnostic methods for the rapid and reliable on-site detection of *Xf* in plant material and in *spy insects*: the direct tissue blot immunoassay (DTBIA) and the real time - loop-mediated isothermal amplification (LAMP) technique. Both techniques are user-friendly, fast and do not require sophisticated equipment or highly skilled operators.

DTBIA or tissue print–ELISA is an accurate serological test whose sensitivity equals that of ELISA; it requires just the imprint of the sample in the field (usually young twigs or stem explants) directly on the nitrocellulose membrane. Printed membranes are processed in the laboratory and a violet color of the prints indicates the positive samples.

An alternative assay to conventional PCR is LAMP, which occurs at isothermal conditions with the use of four specially designed primers, providing a greater level of specificity compared to PCR. The protocol developed by CIHEAM-Bari was applied on-site for the detection of *Xf* through the use of a Smart-DARTTM unit (Diagenetix), a portable device equipped with D&A software that can process six samples each time. The real-time LAMP showed remarkable results in detecting *Xf* mainly from *spy insects*.

XylWeb (D'Onghia *et al.*, 2014)

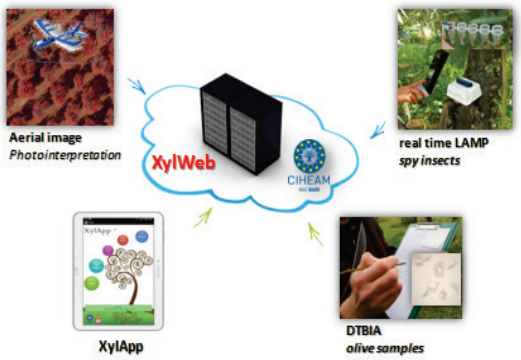
XylWeb is a web server designed and developed by CIHEAM-Bari for the collection, storage and management of the monitoring data flow for *Xf*. This software represents the core of this innovative surveillance system for *Xf* in which all data from the photointerpretation of aerial images, from the field acquisition by XylApp and from pathogen analyses in the laboratory or in the field (DTBIA and real time LAMP) merge by remote transmission.

XylWeb allows data traceability and real time analyses for producing reports and other papers. Its implementation with the regional cartography provides a clear map on the distribution of the samples, infected plants, etc. in order to support the Plant Protection Service in regional decisions for the containment of the infection.

Conclusion

The innovative surveillance system designed for *Xf* is multi-disciplinary, multi-data, multi-functional and multi-actors (Fig.1). Its application, although it is still in progress, is successful for the rapid and continuous monitoring of the pathogen at regional level. This system provides a precise identification of the suspected infected sites to be monitored, the accurate field data acquisition, the rapid onsite pathogen detection avoiding the risk of dissemination of *Xf* and relative vector to "pathogen-free" areas. It represents a valid tool for the Plant Protection Service to locate and properly size the expansion of the infection, and put in place the measures needed to counteract it. Such a model, which integrates different types of techniques and data, could become a permanent system for the surveillance of *Xf* and other invasive harmful pathogens and pests, which may threat the European and Mediterranean countries.

Figure 1
The CIHEAM-MAIB innovative surveillance system for Xylella fastidiosa: multidata, multidisciplinary, multifunctional, multiactors



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