July 2017

Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and vectors.

Xylella fastidiosa is a xylem-restricted pathogen native to the Americas, where it has been confined for long time, and shown to be the causal agent of several detrimental diseases of crops and landscape trees. No cure or therapy treatments have been identified so far to effectively suppress the bacterial population in the infected plants.

The first finding of *X. fastidiosa* in the 'old world' was concomitant with the appearance of a novel and destructive disease on olive trees, reported at the end of 2013 in southern Italy. In this area, the bacterium established itself encountering favorable ecological habitats, susceptible hosts and efficient vector populations. Unfortunately, this first finding has been followed by a series of reports, mainly in France and Spain, which unraveled that *X. fastidiosa* was introduced in the European territory multiple independent times.

In this scenario, effective tools for bacterial detection and identification are essential for monitoring programs and inspections at the borders, for early interception of infected sources/new outbreaks and timely application of preventive and control measures. The objective of the project is to make available rapid and reliable detection protocols for monitoring *X. fastidiosa* in different plant species and associated insect vectors, emphasizing on the harmonization of the protocols routinely adopted in the EPPO region.

During the first year of the project the participants have strengthen collaborations with other EU initiatives dealing with the same phytosanitary threat, i.e. H2020 projects POnTE and XF-ACTORS, with the aim of developing complementary research activities on the diagnosis of the bacterium. A major task developed in this first period was a proficiency test (PT) for the evaluation of the competence of the laboratories carrying-out serological and molecular detection of *X. fastidiosa*. The main objective was to check the ability of 35 different laboratories (from 18 countries) to deliver accurate testing results for the identification of *Xylella fastidiosa* in plant samples, by using serological

(ELISA – enzyme-linked immunosorbent assay) and molecular assays (PCR, real-time PCR). Besides its main scope, this PT gave also the following opportunities: (i) to perform a test performance studies (TPS) for the molecular protocols reported in the EPPO diagnostic protocol PM 7/24 (2) and use the data recovered from the laboratories that were scored as "proficient" (i.e. assessed as 'conform and satisfactory laboratories'); (ii) to train the laboratories with little or no experience on *X. fastidiosa* diagnosis.

The analysis of the data from the proficiency test showed that different laboratories were proficient for different tests selected and described in the EPPO diagnostic protocol PM 7/24 (2).

The next step will be to perform, by the end of 2017, the interlaboratory comparison of molecular tests for the identification of the bacterium in the insect vector *Philaenus spumarius*.

Project ID: Harmonized protocol for monitoring and detection of *Xylella fastidiosa* in its host plants and its vectors. (PROMODE).