# The "Spy Insect" approach for monitoring Xylella fastidiosa in absence of symptomatic plants

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# The “Spy Insect” approach for monitoring *Xylella fastidiosa* in absence of symptomatic plants

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*Xylella fastidiosa*, a vector-borne bacterium, has recently been reported in southern Italy infecting olive trees and more than 28 plant species. The pathogen induces typical leaf scorch and quick decline symptoms; however, many host plants may remain symptomless for years. Its spread by the adults of *Philaenus spumarius* (L.), the only assessed vector, seems very fast due to the poor agronomical practices in the olive groves (e.g. no tillage, no insect control) and to the warm climatic conditions which favour population density and extend the life of infected adults through the whole year. In addition to *P. spumarius*, other 2 insects were reported by Elbeaino *et al.* (2014) as potential vectors because able to harbour *X. fastidiosa*, *Neophilaenus campestris* (Fallén) and *Euscelis lineolatus* (Brullé). The monitoring of the infection in absence of symptomatic hosts in the buffer zone and pathogen-free areas is difficult and requires a randomised sampling for pathogen detection. Due to the quick dissemination of *X. fastidiosa* in Puglia, an effective approach was therefore developed for the early detection of the bacterium in the symptomless areas (D’Onghia *et al.*, 2014). The three Auchenorrhyncha specimens *P. spumarius*, *N. campestris* and *E. lineolatus* are used as ‘spy insects’, i.e. as indicators of the presence of *X. fastidiosa* in apparently uncontaminated areas (Ben Moussa *et al.*, 2016). They have a different seasonal population density which allows the possibility to monitor the pathogen through the whole year. From spring to early autumn, *P. spumarius* followed by *N. campestris* are the most numerous for sampling, while *E. lineolatus* is more frequent in autumn and winter months. A site/submesh is identified and georeferenced, selecting areas with high presence of pathogen host plants. Insects are mainly collected from the ground vegetation or from the host plants using about 10 sweeps with the sweeping net (Fig. 1). However, a D-Vac or yellow sticky traps may be also used but are less efficient. Adults of spy insects are carefully collected by aspiration directly *in loco*, put in small tubes (Fig. 1) containing 70% ethanol, codified and brought to the laboratory for testing and, eventually, identification. If few specimens or no specimen are collected, it is preferred to change the site or combine the collection of 2 sites for a total amount of about 10 adults/site.

The list of the samples and relative code numbers is sent as excel file through XylApp, the application used for field data acquisition (Fig. 1), to the laboratory for analyses and to the central web server, XylWeb (D’Onghia *et al*., 2014).

**a)**

**b)**

**c)**

**Figure 1. a) Aspirator; b) XylApp; c) Sweeping net**

The bacterium is successfully detected in insects by molecular assays (real time PCR and real time LAMP). Nonetheless, real time LAMP is the preferred method because it is fast and accurate; moreover, the use of the field device allows the on-site detection of *X. fastidiosa* in insects and plant material (Yaseen *et al.*, 2015). After results of testing, only the positive insects are identified using the keys of Holzinger *et al.* (2003) classification. Once a positive insect is found, the monitoring of the infection is carried out in a more capillary way in a 100mt radius from the positive sampled site, either collecting plant material from all plant hosts either or capturing other insect's specimens.

The presence of infected insects has two possible explanations: the first one is that the insects have acquired the bacterium from symptomless infected host plants present in the apparently "*Xf*-free" area; the second one is that the insects could have acquired the bacterium in the outbreak area and moved to the pathogen-free area also through indirect transport.

This approach is effective for the early detection of the pathogen in the buffer zone and in the pathogen-free areas. Sampled site for insect captures should be located in the risky points of introduction (e.g. existing trade patterns, traffic ways, nurseries and sites where plants originating in risky areas are grown or kept).

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