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The occurrence of *Xylella fastidiosa* subsp. *pauca* on olive trees in the Salento area (Southern Italy) represent one of the most serious plant health emergencies of recent years in which the entire European Union have had to deal with. A monitoring plan for preventive purposes in the entire national territory was planned by the Italian Ministry of Agriculture by transposing the EU guidelines that foresee the measures to prevent introduction and spread of *X. fastidiosa.* As an example of 'pest-free' area is reported the experience of Latium region, in order to control the sanitary status of the territory and investigate on the presence/absence of *X. fastidiosa.* Taking into account that analyses were mainly focused on asymptomatic plant material, the diagnosis was based on the use of molecular methods characterized by high specificity and sensitivity: real-time PCR. Two assays based on the primers selected on the *rimM* gene and on the primers from the gene encoding the HL protein were used. These methods allowed to exclude the presence of *X. fastidiosa* in the processed samples in spite of the observation of some host plants with suspicious symptoms. An in wide comparison of the adopted approaches, by checking their analytical sensitivity and specificity, showed that the real-time PCR based on *rimM* selected primers was the most accurate for monitoring activity as it does not cause undetermined results when compared to the other real-time PCR (gene encoding HL protein). Other techniques, such as LAMP-PCR, are taken into account to improve the procedures by maintaining the performance of high sensitivity/specificity and in view of direct application in the field.

DETECTION OF *XYLELLA FASTIDIOSA*: VALIDATION AND IMPLEMENTATION OF ROUTINE TESTING METHODS.

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Accurate and early detection of *Xylella fastidiosa* (*Xf*) is a major challenge due to the wide range of host plants (different matrices/tissues, rate of host colonization) and the occurrence of symptomless bacterial infections. The recent establishment of this exotic plant pathogenic bacterium in the EU territory and the large panel of EU susceptible host plants increased the need for rapid diagnostic tools suitable for processing large number of samples and from different sources. Although, several approaches are currently available for the detection of *Xf* in the host plants and vectors, there is a need for harmonized protocols and user-friendly diagnostic tests. In this study, we compared the sensitivity and the reliability of a selected panel of currently available protocols (ELISA, PCR, qPCR), in comparison with novel approaches based on automatized diagnostic platform and on DTBIA and LAMP-based assays. The overall results showed that: (i) although resulting in different diagnostic sensitivity all the approaches tested were able to detect the bacterium in samples from symptomless plants; (ii) Real-time LAMP assay based using crude plant sap can represent a rapid and reliable screening test; (iii) Real-time quantitative PCR assays had the higher diagnostic and analytical sensitivity; (iv) the use of automatized platform allowed to prepare PCR-templates with high and standardized quality for highly reliable diagnostic results; (v) DTBIA had the lowest diagnostic sensitivity, yet representing a useful approach when movement of *Xf* infected materials is limited due to the phytosanitary regulations.

TEST PERFORMANCE STUDY FOR VALIDATION OF DETECTION METHODS OF *XYLELLA FASTIDIOSA*.

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Xylella fastidiosa, a xylem-limited bacterium of the *Xanthomonadaceae* family, was recently associated