RECENT DEVELOPMENTS FOR *XYLELLA FASTIDIOSA* DIAGNOSIS: EVALUATION, VALIDATION AND IMPLEMENTATION OF ROUTINE TESTING METHODS

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The implementation and validation of diagnostic tests included serological and molecular approaches with the aim of pursuing sensitive and reliable identification of the bacterium in a wide range of plant species, including symptomatic and asymptomatic hosts. Serological assays focused on ELISA and DTBIA procedures.
The newly developed polyclonal antisera, raised against the CoDiRO strain, was processed as ELISA and DTBIA reagent, thus evaluated for testing a wide range of plant matrices. Indeed, an *X. fastidiosa* MopB recombinant protein was developed for being used as standardized reference positive control in the diagnostic kits, avoiding the manipulation and the use of bacterial suspension preparations.
In addition, experiments have been made to determine the best procedure for preparing and preserving non-infectious samples (with inactivated bacterium) for diagnostic purposes, including the preparation of the samples to be used for interlaboratory validation and performance test studies. Specifically, freeze-drying and dehydration protocols were used to prepare experimental samples that can be safely manipulated by different diagnostic laboratories without specific quarantine requirements.
For molecular assays, a commercial kit (DNeasy mericon food kit, Qiagen) was successfully adapted for the automatized purification of high-quality DNA from a wide range of host plants. The qPCR assay was implemented by including the simultaneous detection of a plant DNA internal control (multiplex qPCR). A sensitive bacterial detection was also obtained through the development of a real-time LAMP assay based on crude plant sap. All the currently available methods were then tested and compared to a panel of asymptomatic infected plants (olive, oleander, cherry). All approaches were able to identify at a different level the presence of the bacterium, with qPCR assays being the most sensitive tests detecting up to 10^2 CFU/ml.