In France, the detection scheme for plants and vectors is based on the real-time PCR Harper et al. (2010) after a high-throughput DNA extraction based on the QuickPick™ Plant DNA kit used with a KingFisher™ robot. This method was evaluated in 2014–2015, showing excellent performance criteria, apart from a lesser sensibility on olive tree (*Olea europaea*) and some oak species (*Quercus* spp.). In order to improve the limit of detection on these matrices, collaborative works with INRA Angers led to the evaluation of a new protocol based on sample sonication, in order to break *X. fastidiosa* biofilms, CTAB DNA extraction and modified parameters for the real-time PCR mix. Although this method is time consuming, it allows a real improvement of the limit of detection for olive tree and holm oak despite the presence of PCR inhibitors.

Strain identification is performed using MLST scheme (www.pubmlst.org) with the PCR Yuan et al. (2010) for amplifying sequences of 7 housekeeping genes. On plant DNA extracts giving high Ct values with the real-time PCR Harper et al. (2010), some amplification failures were observed. A protocol with addition of BSA into the PCR reaction mixture has been validated, showing improved performance in term of sensibility allowing success for strain typing even in the presence of a small amount of target DNA.

Different approaches for detection of *Xylella fastidiosa* by molecular techniques

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The present work was presented in the framework of the Joint Annual Meeting of the EU Horizon 2020 Projects POnTE 'Pest Organisms Threatening Europe' (GA 635646) and XF-ACTORS 'Xylella fastidiosa *Active Containment Through a multidisciplinary-Oriented Research Strategy' (GA 727987).*

Abstract: The outbreak of *Xylella fastidiosa* in several European countries on many plant hosts make it necessary to have fast, sensitive, and specific methods that allow large-scale surveys in wide areas, in order to know the degree of dispersion of the pathogen. For this purpose, molecular techniques that do or don't require DNA purification have been developed in diagnostic kits by different companies. In this work, a comparative study has been made of some of these kits analying a set of infected almond tree samples from the demarcated area of the outbreak in Alicante (mainland Spain). The real-time PCR by Harper et al. (2010, erratum 2013) and Francis et al. (2006) after manual DNA purification by CTAB was considered the gold standard protocol. First, four different master mixes for real-time PCR were tested, and one of them was selected. Then, the methodologies to be challenged were the automatised DNA extraction with Maxwell® RSC PureFood GMO and Authentication Kit in a Maxwell® RSC instrument (Promega), the Xylella Screen Glow kit based on LAMP technology with the ICGENE system (Enbiotech), the AmplifyRP® XRT+ Isothermal Amplification kit based on recombinase polymerase amplification (Agdia) and phyAlert® kit based on a triplex PCR (MICROGAIA BIOTECH). Interestingly, DNA measurements were not informative of the sensitivity of the protocols challenged.

Similar qualitative results were obtained with all the protocols, each of them with advantages and disadvantages. The choice can be based on the sample size and the economic and human resources of the laboratory. In general, a test with no DNA purification could be used in infected zones for a first screening, further analysing the negative samples with DNA purification previous to real-time PCRs recommended in EPPO 2018 PM 7/24 (3).

Improving *Xylella* sampling in Mallorca

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Abstract: *Xylella fastidiosa* was detected in Majorca in late 2016 (Olmo et al. 2017). Since then, accomplishing EU regulatory, a huge number of samples have been analysed.

Particularly, the Mallorca outbreak is different to others in Europe, because of the coexistence of subspecies *multiplex* (ST81, ST7) in almond trees, olive trees and other species, and subspecies *fastidiosa* (ST1) in almond trees and grapevine plants among others.

As already known, detection tests of *X. fastidiosa* are conditioned by the sampling date. However, it is not well known if the optimal dates for sampling are the same for all host species, if it is viable to pool several samples, or if the bacterium can be detected in old wood samples.

In this study, we have focused the *X. fastidiosa* monitoring on almond, grapevines and olive trees. Monthly percentages of positives and average Ct value of more than 2,000 analyses of each of these crops were compared. Overall, we observed that for almond trees the best results were obtained from samples collected from June to August, whereas in grapevine the optimum period was from August to October. In olive trees early spring resulted in the most favourable time for detection, with an increase in Ct and decrease of positive cases in summer.

In the assays conducted with pooled almond leaf samples, mixing one infected plant extract with the same volume of up to four negative extracts, showed an average Ct increase of 2.7 cycles, whereas for olive and grapevine samples the Ct increase was > 4 cycles.

By default, *X. fastidiosa* is normally analysed in leaf midribs and petioles. We also tested the potential use of wood samples from trunks, sampling different tree rings. Although this sampling procedure is not recommended for routine surveys, it allowed us to establish a potential infection chronology of the number of years since the first infection might have occured in the Balearic Islands.

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Risk-based delimiting survey strategy for *Xylella fastidiosa*

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**Animal and Plant Health Unit (ALPHA), European Food Safety Authority (EFSA)*

Abstract: This poster was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. It provides guidance on the steps of a delimiting survey which are to be followed, upon a new positive finding during a detection survey of *Xylella fastidiosa*. The main objective of a delimiting survey is to establish the boundaries of an area considered to be infested by or free from the pest. The proposed approach is based on a sequence of sampling rounds starting from the periphery, at a certain distance from the centroid of the area to delimit and going towards that centroid. This distance should be based on relevant biological information such as pest spread capacity. Insect vector and plant material should be sampled in the context of the EFSA delimiting survey approach. For both vectors and plants, the sample size is calculated in order for surveillance to achieve an overall 95% confidence level with a 0.5% design prevalence in detecting *X. fastidiosa.* This is done using the statistical tool RiBESS+ which is available online with open access after registration. Following the proposed step-wise approach, an infected zone and corresponding buffer zone can be established.

Risk-based detection survey strategy for *Xylella fastidiosa*

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**Animal and Plant Health Unit (ALPHA), European Food Safety Authority (EFSA)*

Abstract: This poster was prepared in the context of the EFSA mandate on plant pest surveillance (M-2017-0137), at the request of the European Commission. Its purpose is to describe in details the main parameters necessary for a statistically sound detection survey for *Xylella fastidiosa*. In addition, the risk factors relevant for this pest and their relative