M. Bergsma-Vlami, J. L. J. van de Bilt, N. N. A. Tjou-Tam-Sin, C. M. Helderman, P. P. M. A. Gorkink-Smits, N. M. Landman, J. G. W. van Nieuwburg, E. J. van Veen & M. Westenberg, 2017. Assessment of the genetic diversity of *Xylella fastidiosa* in imported ornamental *Coffea arabica* plants. Plant Pathology, doi: 10.1111/ppa.12696

EPPO 2016, PM 7/24 (2) *Xylella fastidiosa*, OEPP/EPPO Bulletin 46 (3), 463–500.

Improving the typing of *Xylella fastidiosa* directly from plant material

Cesbron S. and Marie-Agnès J.*

**INRA, Institut National de la Recherche Agronomique, Beaucouze (FR)*

Abstract: Detection of *X. fastidiosa* directly from plant material is a common practice as a screening test for epidemiosurveillance purposes. MultiLocus Sequence Analysis and Typing (MLSA/MLST) methods are amongst the most widely used genotyping methods for assessing the global epidemiology of various plant pathogenic bacteria including *X. fastidiosa*. MLSA is used to assign strains to one of the known *X. fastidiosa* subspecies and MLST for typing strains and make inference of their origin. Both methods are traditionally used on isolated strains. Nonetheless, because it is time-consuming and sometimes difficult to obtain isolates of *X. fastidiosa* from plant samples as a consequence of an intrinsically poor isolation rate and the fastidious nature of the pathogen, being able to type *X. fastidiosa* in plant samples is a priority for epidemiological purposes. Application of the initial protocol on DNA extracted from plant material proved limited efficiency. The aim of our study was to test the impact of various modifications of the protocol in order to improve the efficiencies of DNA extraction and target amplification rate. We also tested the interest of reduced typing schemes. The direct identification of *X. fastidiosa* infecting plant material face however some limitations that will be discussed.

Acknowledgment
This work was supported by JRC and has received funding from from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE" and grant agreement N. 727987 "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

Bibliography

Denance N, Legendre B, Briand M, Olivier V, de BoissesonC, Poliakoff F & Jacques M-A. 2017. Several subspecies and sequence types are associated with the emergence of *Xylella fastidiosa* in natural settings in France. Plant Pathology doi: 10.1111/ppa.12695.

European Plant Protection Organization, 2016. EPPO Standards PM 7 – Diagnostics PM 7/24 (2) *Xylella fastidiosa*. Bulletin OEPP/EPPO Bulletin 46, 463–500.

Real-time LAMP rapid diagnostic method for *X. fastidiosa* in plant material and insect vectors

Yaseen T.*, Drago S., Stampone G., D'Onghia A. M:

**CIHEAM Mediterranean Agronomic Institute of Bari, Valenzano, Bari (IT)*

Abstract: *X. fastidiosa* is a gram-negative insect-vectored bacterium has been recently detected in Italian olive trees severely affected by Olive Quick Decline Syndrome (OQDS). ELISA tests and Polymerase Chain Reaction (PCR) assays were largely used in the monitoring campaign of this pathogen. The aim of this study is to evaluate the new real time Loop-mediated isothermal amplification (LAMP) system for the detection of *X. fastidiosa* in host plants and insect vectors. The new detection system is composed of a portable instrument (icgene mini) and a ready to use diagnostic kit denominated "*Xylella* Screen Glow" (Enbiotech s.r.l.- Italy). Specificity and sensitivity of *Xylella* Screen Glow kit was compared with PCR and real-time quantitative PCR assays. Three different DNA extraction protocols and typologies of infected materials were also tested. The real-time LAMP system showed high specificity and a sensitivity as compared to the real-time qPCR and PCR assays. These results showed that "*Xylella* Screen Glow" kit with icgene portable LAMP instrument is highly

sensitive and suitable for *X. fastidiosa* detection directly in the field. Therefore, this system could be applied on site, with high sensitivity and readability to prevent the movement of infected materials from *X. fastidiosa* contaminated area to the laboratories located in free areas; other possible application could be in quarantine station to intercept any infected material before crossing border and protect from a country from such dangerous quarantine pathogen.

Acknowledgment
This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

Bibliography

YASEEN, Thaer et al. On-site detection of Xylella fastidiosa in host plants and in "spy insects" using the real-time loop-mediated isothermal amplification method. Phytopathologia Mediterranea, [S.l.], v. 54, n. 3, p. 488-496, sep. 2015. ISSN 1593-2095. Date accessed: 20 May. 2017. doi:10.14601/Phytopathol_Mediterr-15250.

Session 7 – From field detection to disease dynamics

Detecting *X. fastidiosa* with hyperspectral remote sensing: findings from two years of airborne campaigns in Puglia

Zarco-Tejada P.J.*, Calderon R., Beck P.S.A., González-Dugo V., Montes-Borrego M., Hornero A., Landa B.B., Boscia D., Susca L., Navas-Cortes J.A.

**Joint Research Centre, European Commission*

Abstract: A remote sensing campaign carried out in Puglia in summer 2016 collected hyperspectral and thermal images of ca 200,000 olive trees at sub-meter resolution. In the 1200 ha study area within the *X. fastidiosa* infected zone, 3500 trees were simultaneously evaluated in the field for severity of *X. fastidiosa* symptoms. The hyperspectral sensor used in this experiment acquired data in the visible and near-infrared spectral region (400-885 nm) with 260 bands of 6.5 nm FWHM at 1.85 nm/pixel and 12-bit radiometric resolution. The sensor was radiometrically calibrated in the laboratory, and images atmospherically corrected to obtain surface reflectance using total incoming irradiance measured in the field. The high resolution hyperspectral and thermal imagery acquired over the orchards allowed the delineation of individual trees using object-based crown detection algorithms. Crown temperature and hyperspectral indices were calculated for each tree to classify disease severity levels using different machine learning algorithms, including linear discriminant analysis, support vector machines and neural networks. The success and applicability of these early detection methods to other areas will be discussed in the context of a new airborne campaign planned in July 2017 over *X. fastidiosa* infected zones in Mallorca. In this new airborne campaign the impact of *X. fastidiosa* symptoms on crown reflectance will also be evaluated in the 800-1700 nm spectral region.

Acknowledgment
This work was supported by JRC and has received funding from from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 635646 "Pest Organisms Threatening Europe POnTE" and grant agreement N. 727987 "*Xylella fastidiosa* Active Containment Through a multidisciplinary-Oriented Research Strategy XF-ACTORS".

Preliminary results on the canopy distribution of *Xylella fastidiosa* in the Apulian olive cultivars

Santoro F., Valentini F., Frasheri D., Gualano S., Yaseen T., D'Onghia A.M.*

**CIHEAM Mediterranean Agronomic Institute of Bari, Valenzano, Bari (IT)*

Abstract: Pathogen detection in the Italian outbreak area of *X. fastidiosa* is carried out using samples from symptomatic olive twigs and leaves. Conversely detection from asymptomatic trees is difficult due to the uneven pathogen distribution in olive cultivars. In this study the incidence and canopy