Disease Note

XYLELLA FASTIDIOSA IN *COFFEA ARABICA* ORNAMENTAL PLANTS IMPORTED FROM COSTA RICA AND HONDURAS IN THE NETHERLANDS

M. Bergsma-Vlami, J.L.J. van de Bilt, N.N.A. Tjou-Tam-Sin, B.T.L.H. van de Vossenberg and M. Westenberg

Dutch National Plant Protection Organization (NPPO-NL), P.O. Box. 9102, 6700 HC Wageningen, The Netherlands

The European Union (EU) has recently implemented annual surveys in its member States, in order to prevent further introductions of the harmful organism *Xylella fastidiosa*, within its territory, upon an outbreak in southern Italy where this quarantine pathogen has been identified as the putative agent of the Olive quick decline syndrome (OQDS), (Boscia, 2014; EFSA, 2015). *X. fastidiosa* affects several economically important plants reported mainly from the Americas, including grapevine, stone fruits, coffee and citrus and is the causal agent of leaf scorch disease of many ornamentals, shade and forest trees. *X. fastidiosa* subsp. *pauca* has also been associated in Costa Rica with a coffee disease known as "crespera", characterized by irregular leaf growth, atypical curling of the leaf margins, reduced leaf size, shortening of the internodes and severe chlorotic mosaic of the leaves (Montero-Astúa *et al*., 2008). *X. fastidiosa* subsp. *pauca* and *X. fastidiosa* subsp. *fastidiosa* were recently intercepted and diagnosed in imported *Coffea arabica* plants from Equador and *C. canephora* from Mexico. These plant consignements have been destroyed (EPPO, 2012; Legendre *et al*., 2014). *X. fastidiosa* was presumptively diagnosed during a survey performed on ornamental *C. arabica* plants imported from Costa Rica and Honduras into the Netherlands in the autumn of 2014, as part of the recent EU implementing decision (Commission Implementing Decision 2014/87/EU). These plants showed either mild leaf scorch symptoms, or a range of "crespera"-like symptoms, or no symptoms. This presumptive diagnosis of *X. fastidiosa* was in agreement with EPPO's (2004) recommendations and was carried out with molecular and serological methods. Total genomic DNA was extracted from leaf veins and petioles of symptomatic and latently infected *C. arabica* plants with the QuickPic SML plant DNA kit (Bio-Nobile, Finland), using a KingFisher isolation robot (Thermo Scientific, The Netherlands). Real-time PCR on purified genomic DNA using Premix ExTaq (TaKaRa, France) gave positive reactions using the *X. fastidiosa* gene-specific primers targeted to 16S rRNA-processing gene *rim*M (Harper *et al*., 2010, erratum 2013). Conventional PCR using GoTaq polymerase (Promega, The Netherlands) and primer set RST 31/RST 33 (Minsavage *et al*., 1994) confirmed the presumptive presence of *X. fastidiosa* in these samples. Sequence analysis of the *ca*. 700 bp amplicon of the RNA polymerase sigma 70 factor showed 96 to 100% identity with the comparable genomic regions of *X. fastidiosa* strain sequences from GenBank, which indicated the presence of *X. fastidiosa* DNA in the plant extract. Remarkably, three different sequences with 97-98% identity among each other (GenBank accession Nos. KP769842-KP769844) were recovered from imported *C. arabica* plants. Two of these sequences were related to *C. arabica* plants imported from Costa Rica, whereas the third sequence was only present in plants imported from Honduras. Extracts from leaf veins and petioles were exposed to indirect immunofluorescence (IF), using a commercially available antiserum (Loewe 07319, Germany). IF ascertained the presence of *X. fastidiosa,* thus confirming the molecular tests. Final diagnosis, based on the recovery of the *C. arabica* infecting isolate(s) of *X. fastidiosa* in axenic culture, for evaluation of its pathogenicity and subspecies identification is currently in progress.

Corresponding author: M. Bergsma-Vlami
E-mail: m.vlami@nvwa.nl

Received March 10, 2015
Accepted March 19, 2015

Boscia D., 2014. Occurence of *Xylella fastidiosa* in Apulia. *Proceedings of the International Symposium on the European outbreak of Xylella fastidiosa in olive, Gallipoli-Locorotondo, Italy, 21-24 October 2014*, p. 30.

2014/87/EU: Commission Implementing Decision of 13 February 2014 as regards measures to prevent the spread within the Union of *Xylella fastidiosa* (Well and Raju) (notified under document C(2014) 726).

EFSA PLH Panel (EFSA Panel on Plant Health), 2015. Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options. *EFSA Journal* **13**: 3989, 262 pp., doi: 10.2903/j.efsa.2015.3989.

EPPO, 2004. Diagnostic protocols for regulated pests. *Xylella fastidiosa*. Bulletin *OEPP/EPPO Bulletin* **34**: 187-192.

EPPO, 2012. *Xylella fastidiosa* detected in a containment facility in France. *EPPO Reporting Service*, **No 8**. 2012/165.

Harper S.J., Ward L.I., Clover G.R.G., 2010 (erratum 2013). Development of LAMP and Real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology* **100**: 1282-1288.

Legendre B., Mississipi S., Oliver V., Morel E., Crouzillat D., Durand K., Portier P., Poliakoff F., Jacques M.A., 2014. Identification and characterisation of *Xylella fastidiosa* isolated from Coffee plants in France. *Journal of Plant Pathology* **96**: S4.100

Minsavage G.V., Thompson C.M., Hopkins D.L., Leite R.M.V.B.C., Stall R.E., 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. *Phytopathology* **84**: 456-461.

Montero-Astúa M., Chacon-Diaz C., Aguilar E., Rondriguez C.M., Garita L., Villalobos W., Moreira L., Hartung J.S., Rivera C., 2008. Isolation and molecular characterization of *Xylella fastidiosa* from coffee plants in Costa Rica. *Journal of Microbiology* **46**: 482-490.