**Disease Note**

**Xylella Fastidiosa in Coffea Arabica Ornamental Plants Imported from Costa Rica and Honduras in the Netherlands**

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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 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 consignments 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 (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 *rsmM* (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.


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).


