New Disease Reports

First report of '*Candidatus* phytoplasma ulmi' (16SrV-A) associated with *Ulmus* cultivar Morfeo (elm) in the United Kingdom

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Young trees of *Ulmus* (elm) hybrid cultivar Morfeo were imported into a commercial nursery in the south of the United Kingdom from Italy. Approximately one year after importation the trees began to show symptoms typical of phytoplasma infection, primarily pronounced foliar proliferation and stunting. DNA was extracted from 0.3 g of leaves or roots from five symptom-bearing trees using a CTAB method (Doyle & Doyle, 1990) and was tested by real-time PCR using the universal phytoplasma assay (Hodgetts *et al*., 2009), all being found to be positive. Three samples were randomly selected for further analysis to identify the phytoplasma present. Nested PCR of the 16S rRNA gene was performed using universal phytoplasma primers P1 and P7, followed by R16F2n and R16R2 as described in Hodgetts *et al*. (2007), where the samples produced the specific 1,250 bp amplicon. The PCR amplicon was cloned into the pGEM®-T Easy Vector System (Promega, USA), three randomly selected clones sequenced and the sequence was submitted to NCBI (GenBank Accession No. KT381877). Phylogenetic analysis was undertaken which identified the phytoplasma as a member of the 16SrV phytoplasmas (Fig. 1), but did not provide adequate discrimination to the species level.

To further discriminate the phytoplasma, conventional PCR to amplify the *secY* and elongation factor Tu (*tuf*) genes were performed as described in Foissac *et al*. (2013). The presence of a phytoplasma was confirmed by the amplification of the expected amplicon for both phytoplasma genes in all of the trees sampled. DNA sequencing was undertaken for both genes and the sequence was submitted to NCBI (KT381878 for *secY* and KT381876 for *tuf*) and phylogenetic analysis undertaken. Both genes indicated the phytoplasma to be a member of '*Candidatus* Phytoplasma ulmi', group 16SrV-A (Figs. 2, 3).

This is the first report of '*Ca.* P. ulmi' in elm in the UK, a highly significant finding as it is an EPPO (European and Mediterranean Plant Protection Organization) A1 listed organism. Therefore all plants from the importation have been destroyed, and a wider survey of elm has been undertaken to identify if earlier imports have allowed the establishment of'*Ca.* P. ulmi' within the UK. Follow-up surveys for visual symptoms were carried out in nurseries stocking *Ulmus* and recently planted sites across the UK and Channel Islands. Over 11,000 trees were inspected and no symptoms typical of phytoplasma infection were observed. A survey of the UK National Elm Collection was also undertaken which included the testing of over 50 asymptomatic trees by real-time PCR; all were found to be negative for the presence of phytoplasmas. Therefore this is believed to be an isolated importation and the phytoplasma has been eradicated from the UK.

Acknowledgements

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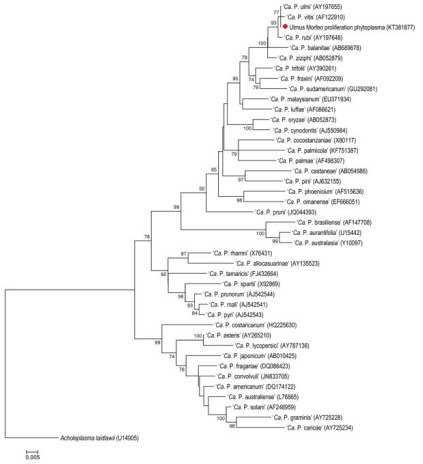

Figure 1

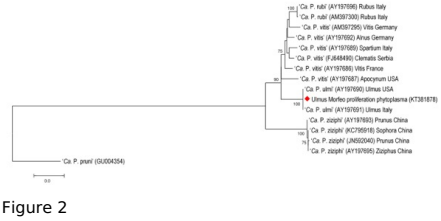

Figure 2

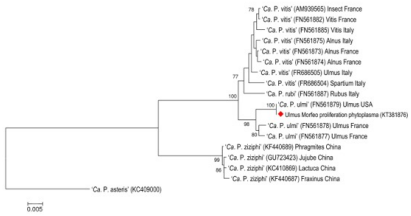

Figure 3

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