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DISEASE NOTES

First Report of *'Candidatus* Phytoplasma trifolii'-Related Strain of 16SrVI-A Phytoplasma Subgroup, Associated with Elm Yellows Disease in American Elm (*Ulmus americana* L.) in Ohio, U.S.A

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During the investigation of the sudden and early onset of yellowing, followed by mortality of American elm (*Ulmus americana* L.) trees at the USDA Forest Service Northern Research Station in Delaware, Ohio, a phytoplasma of the clover proliferation group (16SrVI) was detected as the putative causal agent of the disease outbreak. Onset of symptoms was rapid and widespread, occurring in late July 2016 and affecting ~60 trees across two elm research plantations. Symptoms included a general yellowing of individual tree canopies, epinasty of foliage throughout the canopy, phloem discoloration, and on a subset of trees, a strong odor of methyl salicylate (observed in phloem tissue extracted from the lower stem). Similar symptoms in elms have been attributed to the classic elm yellows '*Candidatus* Phytoplasma ulmi' (16SrV-A) (Lee et al. 2004) and the Illinois elm yellows phytoplasma (16SrVI-C) (Jacobs et al. 2003). In July 2016, samples were collected from 12 symptomatic and 8 asymptomatic American elm trees. DNA from the leaf midrib and branch phloem was isolated and analyzed for phytoplasma via seminested polymerase chain reactions (PCR). PCRs were first primed by phytoplasma universal primer pair P5/P7 (Jomantiene et al. 1998), followed by P7 and the reverse complement of the universal phytoplasma primer R16R2 for amplification of the phytoplasma 16S-23 ribosomal (r) DNA (16S-23 rRNA gene) sequences as per Gundersen and Lee (1996). The predicted band size of the second PCR product is 487 base pair (bp). The product bands were isolated, purified, and sequenced using primer P7. Sequencing results of the PCR products indicated that nine of the symptomatic and one of the asymptomatic American elm trees tested were infected by a phytoplasma. A BLAST search of the DNA sequences indicated high similarities to members of the '*Ca*. P. trifolii' group 16SrVI-A. The sequences of all 10 phytoplasma-infected trees were identical to each other. To further confirm that a strain of '*Ca*. P. trifolii' was infecting the elms, a PCR product was cloned and sequenced. The 1,557 bp band was the product of primers P1a/P7, followed by PCR primers designed from the sequence of '*Ca*. P. trifolii'. This band was cloned into the pMiniT 2.0 vector. Plasmid sequencing used standard sequencing primers (SP6 and T7 promoters), the primers included with the vector, and finally, custom designed phytoplasma primers that eliminate other bacterial DNA from getting amplified. The entire plasmid clone was sequenced in both directions with four to six times of coverage per base. Determination of the phytoplasma classification group was based on the nucleotide sequence within the phytoplasma universal primers F2n/R2 PCR fragment within the 16Sr gene. Using *i*PhyClassifier, the online tool for phytoplasma classification and taxonomic assignment (https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi), the sequence similarity

between the Delaware elm phytoplasma and '*Ca.* P. trifolii' (GenBank accession no. AB279597.1) is 99.9% in the 16Sr region, which places the Delaware elm phytoplasma in the 16SrVI-A group. The sequence of the plasmid was deposited in GenBank under accession number MF385584. Elm decline and yellows diseases in North America have been associated with the Illinois elm yellows phytoplasma (16SrVI-C, GenBank accession no. AF268893.1) (Jacobs et al. 2003), '*Ca*. Phytoplasma ulmi' (16SrV-A) (Lee et al. 2004), and phytoplasma in the aster yellows group (16SrI-C) (Lee et al. 1995).

References

Gundersen, D. E., and **Lee, I.-M.** 1996. Phytopathol. Mediterr. 35:144.

Jacobs, K. A., et al. 2003. Plant Dis. 87:241. https://doi.org/10.1094/PDIS.2003.87.3.241 [Abstract] [ISI]

Jomantiene, R., et al. 1998. HortScience 33:1069. [ISI]

Lee, I.-M., et al. 1995. Phytopathol. Mediterr. 34:174.

Lee, I.-M., et al. 2004. Int. J. Syst. Evol. Microbiol. 54:337. https://doi.org/10.1099/ijs.0.02697-0 [Crossref] [ISI]