Diseases Caused by Bacteria and Phytoplasmas

Olive Tree Represents a New Host of a Subgroup 16SrVII-B Phytoplasma Associated with Witches' Broom Disease in Brazil

J. Ferreira, T. B. C. Pereira, C. A. Almeida, and I. P. Bedendo[†]

Department of Plant Pathology and Nematology, ESALQ, University of São Paulo, Piracicaba, SP, Brazil

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Olive trees exhibiting slow development, yellowing, and high intensity of shoot proliferation with small leaves were observed in commercial plantings, in the municipality of Extrema, Minas Gerais (MG) state in 2015. The incidence of symptomatic plants was about 70%, and diseased trees presented yield reduction. Here we report the association of symptomatic olive trees with a phytoplasma and describe its molecular identification. Symptomatic plants (38 trees) were sampled in three growing areas located in the same municipality. The samples consisted of a bunch of leaves and young shoots. The total DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Phytoplasma detection was conducted by nested PCR with primers P1/16S-SR (Lee et al. 2004) followed by R16F2n/R16R2 (Gundersen and Lee 1996). PCR assays generated amplicons (~1.2 kb) from 28 trees out of 38 symptomatic plants, confirming the association of phytoplasma with diseased plants. The disease was named olive witches' broom. The genomic fragments amplified by nested PCR were cloned into *Escherichia coli* DH5 α and sequenced. The sequence representative of the olive phytoplasma was designated OWB-Br01 (Olive Witches' Broom-Brazil 01) and deposited in GenBank under accession number MH141985. This sequence shared 99% sequence identity with phytoplasmas affiliated with 16SrVII group. According to the *iPhyClassifier* online tool (Zhao et al. 2009) the olive witches' broom phytoplasma was classified as a variant of subgroup 16SrVII-B with a pattern similarity coefficient of 0.99. The phylogenetic tree showed that OWB-Br01 phytoplasma emerges from the same branch of the reference phytoplasma of the 16SrVII-B subgroup (Erigeron witches'-broom phytoplasma,

GenBank AY034608), indicating that the olive tree phytoplasma is a member of the 16SrVII-B subgroup. The pathogenicity test was performed with 28 healthy plants (cultivar Arbequina) grown in pots, which were grafted by simple English forklift with scions obtained from olive plants (Arbequina) that were 6 years old, naturally infected by the phytoplasma. The initial symptoms were observed 4 months after grafting, and at 8 months 22 grafted plants exhibited slow growth, yellowing, and small leaves as those naturally observed in the fields. Molecular characterization allowed identifying the phytoplasma as a member of the 16SrVII-B subgroup. In Brazil, representatives of the 16SrVII group were previously reported in association with diverse botanical species. Thus, a strain of 16SrVII-C subgroup was identified in sunn hemp (Flôres et al. 2013), the reference phytoplasma of 16SrVII-D subgroup was found in erigeron plants (Flôres et al. 2015), and the representative of 16SrVII-F was detected in the wild species *Vernonia brasiliensis*. (Fugita et al. 2017). Specifically regarding subgroup 16SrVII-B, the reference phytoplasma of this subgroup was described from erigeron and periwinkle (Barros et al. 2002), whereas other members of this subgroup were reported in cauliflower (Pereira et al. 2016a) and ming aralia (Pereira et al. 2016b). The disease here studied is a threat because olive planting is in large expansion in Brazil. A potential control option could be use of propagative material from sources free of the pathogen. Based on our findings, olive tree represents a new host for subgroup 16SrVII-B phytoplasma, which is different from 16Sr groups previously reported as associated with olive witches' broom in other countries.

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[†]Indicates the corresponding author.

E-mail: I. P. Bedendo; ibedendo@usp.br