Diseases Caused by Oomycetes

First Detection of Leaf Blight and Stem Canker Caused by *Phytophthora ramorum* on Brisbane Box in the United States

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Brisbane box, *Lophostemon confertus* (Myrtaceae) is a frost-tender evergreen tree planted for its upright form, large ovate leaves, and attractive white flowers that bloom in the spring. In June 2017, the Plant Pest Diagnostics Center lab received a call from an arborist who described Brisbane box street trees dying in central Sausalito, Marin Co., California. Trees ranged from containing 10% to nearly 80% dead hanging leaves. Six trees along the same street were affected. Wilted brown leaves remained attached to branchlets covered in black cankers. Some healthy branchlets had leaves with angular spots that crossed the veins and were surrounded by yellow halos. Isolations were made onto corn meal agar amended with pimaricin, ampicillin, rifampicin, and pentachloronitrobenzene (CMA-PARP) (Jeffers and Martin 1986) from the canker and leaf spot margins. A *Phytophthora* species resembling *Phytophthora ramorum* grew on CMA-PARP medium with coraloid coenocytic hyphae, chlamydospores, and ellipsoidal semipapillate sporangia. The internal transcribed spacer region (ITS) of rDNA was amplified and sequenced using primers PHY.OO.18S.F and PHY.OO.28S.R (Rooney-Latham et al. 2019). BLAST analysis of 770 base pairs of the sequenced amplicon (GenBank MK993541) showed 100% identity with the ITS sequence of the *P. ramorum* ex-type (MG865581). A portion of the *cox2* gene was amplified and sequenced (Hudspeth et al. 2000) (GenBank MK994528), and 530 base pairs matched with 100%

identity (GU222130). Pathogenicity was confirmed by inoculating three initially 1.8-m-tall trees in 18.9-liter pots. Prior to inoculations, trees were cut so they would fit into 122-cm-high dew and growth chambers. For each tree, three lower branchlets measuring from 4 to 10 mm in diameter were inoculated by wounding with a 6-mm punch, placing a colonized agar plug in the wound, and then wrapping with Parafilm. Lower branches were covered in plastic to protect them from subsequent zoospore inoculation. Branchlet inoculum was prepared by growing *P. ramorum* on V8 juice agar (V8) for 4 days at 22°C. Zoospores were prepared for leaf inoculation by taking 6-mm agar plugs from the margin of 6-day-old cultures and flooding plugs in soil water for 4 days. Zoospores were released by transferring plugs to sterile distilled water at 4°C for 1.5 h. Leaves on the same three trees that were inoculated with the plugs were sprayed with 350 ml of zoospores (2×10^5 zoospores/ml) and placed in a dew chamber at 23°C for 48 h. Afterward, they were transferred to a growth chamber (23°C, 12-h diurnal cycle), where the plastic was removed from the lower branches after leaves had dried. A single control tree was treated similarly with uncolonized V8 plugs, followed by a water spray. Leaf spots were visible 4 days later, with inoculated leaves turning necrotic and abscising after 3 weeks. Cankers from inoculated branchlets measured from 12 to 60 mm long after 60 days. *P. ramorum* was isolated from the margin of every inoculated canker and leaf spot. No *P. ramorum* was isolated from the control tree. To our knowledge, this is the first report of *P. ramorum* on *L. confertus* in the world. Natural inoculum presumably came from infected *Umbellularia californica* trees located less than 800 m west of the trees in Sausalito. This detection will further limit the planting choices of arborists and landscapers in *P. ramorum*-infected locations.

References:

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