Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Laurel Wilt Disease Caused by *Raffaelea lauricola* on Sassafras in Virginia

R. Gazis,¹ K. M. DeWitt,² L. K. Johnson,² L. A. Chamberlin,² A. H. Kennedy,³ M. A. Hansen,⁴ and E. A. Bush^{4,†}

¹University of Florida, Homestead, FL 33031

²Virginia Department of Forestry, Charlottesville, VA 22903

³National Identification Services-PPQ-APHIS-USDA, Beltsville, MD 20705

⁴Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

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Laurel wilt is a lethal vascular disease affecting native Lauraceae in North America. The causal fungus, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva, and its symbiont, redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, are native to Asia (Fraedrich et al. 2008; Harrington et al. 2008). Since their introduction near Savannah, Georgia, in 2002 (Fraedrich et al. 2008), laurel wilt has spread rapidly, resulting in extensive mortality of native redbay (*Persea borbonia* [L.] Spreng.) (Hughes et al. 2017), and is a threat to other native Lauraceae, such as sassafras (*Sassafras albidum* [Nutt.] Nees) (Bates et al. 2013) and northern spicebush (*Lindera benzoin* [L.] Blume) (Olatinwo et al. 2021). In June 2021 a sassafras sapling showing wilt and dieback was observed along a roadside in Scott County, Virginia, which borders a laurel wilt-positive Tennessee county (Lloyd et al. 2020). The trunk (approximately 5 cm diameter) was submitted to the Virginia Tech Plant Clinic. Although beetle holes were observed, *X. glabratus* was not found. Discolored sapwood chips were excised and plated on malt extract agar amended with cycloheximide (200 ppm) and streptomycin (100 ppm) (CSMA). A fungus was consistently recovered, and the morphology of conidiophores and conidia and presence of blastoconidia and mucoid growth aligned with the description of *R. lauricola* (Harrington et al. 2008). Two *R. lauricola*-specific primer sets (Dreaden et al. 2014) were used to amplify DNA extracted from a representative isolate (0248-2021) and confirm *R. lauricola*. For further

confirmation, the LSU region of the rDNA was sequenced (Lloyd et al. 2020). The sequence of the isolate (GenBank accession no. OL583842) showed 100% identity (573/573 bp) to *R. lauricola* ex-type strain sequence, CBS 121567 (accession no. MH877762) (Harrington et al. 2008; Vu et al. 2019). The isolate was also confirmed by the National Identification Services by sequencing. To confirm pathogenicity, 15 sassafras seedlings (height = 60 to 100 cm, diameter = 8 to 10 mm) were inoculated with a conidial suspension harvested from 10-day CSMA cultures of 0248-2021, as follows: two 0.4 mm diameter holes were drilled 10 cm above the soil line at a 45° angle on opposite sides of the stem, leaving at least 3 cm between holes. Then, 10 µl of the conidial suspension (5×10^7 /ml) was transferred into each hole and sealed with Parafilm. Two sassafras seedlings were inoculated with sterile water. Seedlings were maintained with 12 h photoperiod at 27° ± 2°C. Off-color foliage and loss of turgor were observed 10 days postinoculation on conidia-inoculated seedlings; at two weeks, these were completely wilted and had sapwood discoloration. Water-inoculated plants showed no symptoms. Sapwood from 15 cm above the inoculation point was excised from 0248-2021-inoculated plants ($n = 2$) and water-inoculated plants ($n = 1$) and plated on CSMA. *R. lauricola* was recovered from symptomatic plants, but not from water-inoculated plants. The identity of the recovered fungus was confirmed with two species-specific primer sets (Dreaden et al. 2014). It is likely that laurel wilt is prevalent in the area of the roadside find. Both sassafras and northern spicebush are widespread in Virginia and their range extends into the northeastern US and lower Canada. Laurel wilt poses a serious threat to these species and their ecosystems. For example, spicebush and sassafras are primary hosts of the native spicebush swallowtail butterfly (*Papilio troilus* L.) (Nitao et al. 1991).

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[†]Indicates the corresponding author.
E. A. Bush; ebush@vt.edu