Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Laurel Wilt Disease Caused by *Raffaelea lauricola* on Spicebush in Louisiana

R. Olatinwo,^{1,†} J. Hwang,² and W. Johnson²

¹USDA Forest Service, Southern Research Station, Pineville, LA 71360

²USDA Forest Service, Forest Health Protection, Pineville, LA 71360

Plant Dis. 105:2250, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2511-PDN>. Accepted for publication 26 February 2021.

In the past two decades, laurel wilt disease has significantly affected members of the Lauraceae in the southeast United States, causing widespread mortality of native redbay (*Persea borbonia* [L.] Spreng) and incidence of infections in avocado (*Persea americana* Mill.), sassafras (*Sassafras albidum* L.), and swamp bay (*Persea palustris* [Raf.] Sarg.) (Fraedrich et al. 2008, 2015; Olatinwo et al. 2019). Laurel wilt is a vascular disease caused by *Raffaelea lauricola* (T.C. Harr., Fraedrich & Aghayeva), a fungus vectored by a nonnative ambrosia beetle *Xyleborus glabratus* Eichhoff (Fraedrich et al. 2008). In August 2020, we investigated the mortality of a spicebush shrub (*Lindera benzoin* L.) (3.8 cm diameter at root collar, 2 m height) located ~17 miles northeast of Colfax, Grant Parish, Louisiana (31.750263° N, -92.643694° W). Evaluation of the dead shrub revealed brown, persistent foliage and black vascular discoloration of the sapwood, typical symptoms of laurel wilt. Although beetle holes were observed on the sapwood, no beetle was found in galleries at the time. In the laboratory, a fungus consistently isolated from surface-sterilized sapwood tissues plated on potato dextrose agar (PDA) was identified as *R. lauricola* based on the morphological characteristics of the isolate (i.e., mucoid growth, conidiophores, and oblong/ovoid shape conidia [Harrington et al. 2008]). The fungal isolate was denoted as SB1. The identity of the fungus was confirmed by positive PCR amplification of the large subunit ribosomal RNA gene region using species-specific primers rab-lsu-rl_F (CCCTCGCGGCGTATTATAG) and rab-lsu-rl_R (GCGGGGCTCCTACTCAAA) (Olatinwo, unpublished). The sequence of isolate SB1 (GenBank accession no. MW207371) showed

100% homology to the *R. lauricola* strain CBS 127349 sequence (GenBank accession no. MH877933). The pathogenicity of SB1 on spicebush was evaluated on four healthy shrubs (average 1 m height and 40 mm in diameter) at the location from which the original detection was made. Stems of two spicebush shrubs were inoculated with SB1 agar plugs from a 14-day-old culture on PDA, whereas plain PDA plugs were used on the remaining two shrubs as noninoculated controls. Agar plugs were placed in 5 mm (0.2 in) diameter holes punched on the bark with a cork borer as described by Mayfield et al. (2008). After 6 weeks, the *R. lauricola*-inoculated shrubs were wilted with noticeable blackened tissue discoloration in the sapwood, whereas the control trees remained healthy. *R. lauricola* was reisolated from tissue of the two inoculated symptomatic shrubs but not from the control trees. The sequence of the reisolated *R. lauricola* isolate, denoted as SB3 (GenBank accession no. MW207372), showed 100% homology to the *R. lauricola* strain CBS 127349 and isolate SB1. This first documentation of laurel wilt on spicebush in Louisiana is significant because spicebush berries, leaves, and twigs are food sources for forest animals, birds, and insects including whitetail deer and spicebush swallowtail (*Papilio troilus* L.). Since its first report on sassafras in 2014 (Fraedrich et al. 2015), laurel wilt has spread across Louisiana on sassafras and swamp bay (Olatinwo et al. 2019) and has been confirmed in 14 parishes. This report shows the relentless nature of the disease, as the pathogen moves from one vulnerable host to the next, expanding into new locations and threatening forest ecosystems across the southern United States.

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The author(s) declare no conflict of interest.

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Keywords: spicebush, laurel wilt, *Raffaelea*, *Lindera*, vascular disease

[†]Indicates the corresponding author.
R. Olatinwo; rabi.u.o.latinwo@usda.gov