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

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Abstract

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 non-native 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, two m height) located ca. 17 mi 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 (Fig. S1). 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region using species-specific primers; rab-lsu-rl_F: CCCTCGCGGCGTATTATAG and rab-lsu-rl_R: GCGGGGCTCCTACTCAA (Olatinwo, unpublished). The sequence of the 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, while plain PDA plugs were used on the remaining two shrubs as non-inoculated controls. Agar plugs were placed in 5 mm (0.2 in) diameter hole punched on the bark with cork-borer as described by Mayfield et al (2008). After six weeks, the *R. lauricola* inoculated shrubs were wilted with noticeable blackened tissue discoloration in the sapwood, while the control trees remained healthy (Fig. S2). *Raffaelea lauricola* was re-isolated

from tissue of the two inoculated, symptomatic shrubs, but not from the control trees. The sequence of the re-isolated *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.

Keywords: Crop Type; Epidemiology; Pathogen detection; Subject Areas; Trees; disease development and spread; forest; tree.