



Field and Growth Chamber Inoculations Demonstrate *Persea indica* as a Newly Recognized Host for the Laurel Wilt Pathogen, *Raffaelea lauricola*

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Laurel wilt, caused by the fungus *Raffaelea lauricola*, is a vascular disease of native and exotic trees in the Lauraceae in the USA (2). Recently, a study was conducted to investigate the attractiveness of *Persea* species to the laurel wilt vector, *Xyleborus glabratus* (redbay ambrosia beetle) (3). Hung bolts of the tree species *P. indica* were shown to be as or more attractive to *X. glabratus* than *Persea borbonia* (redbay), its primary host in the USA (3). In order to assess the susceptibility of *P. indica* to the laurel wilt fungus, field and growth chamber experiments were conducted. In May 2010, 12 trees of *P. indica* were planted at the University of Florida Plant Sciences Research and Education Unit in Citra, FL, and 10 were inoculated with 1.0×10^5 conidia of *R. lauricola* isolate RL4 (CBS 127349; GenBank accession no. HM446155) as described previously (4), with two trees serving as water-inoculated controls. After 6 weeks, all inoculated plants displayed symptoms of laurel wilt similar to those that develop on *Persea americana* (avocado), including wilt, defoliation, leaf necrosis, shriveling of small branches, and brown/black discoloration of sapwood. *R. lauricola* was consistently isolated from symptomatic sapwood (2,4).

In March 2012, six containerized seedlings of *P. indica*, two of *Persea palustris* (swamp bay), a preferred host of *X. glabratus* and *R. lauricola*, and a *Quercus alba* (white oak) non-host control were placed in a growth chamber (25°C, 12:12 diurnal light). On all plants, bark was scored with a 3-mm cork-borer, and to a single *P. indica* and *P. palustris* plant 3 empty gelatin capsules were attached to the lower stem (mock-inoculated controls). On the remaining *P. indica* plants and one each of *P. palustris* and the *Q. alba*, a single female of *X. glabratus* (reared from an infected redbay log) within an individual gelatin capsule with ventilation holes was affixed to the lower stem, with 3 capsules/beetles per plant. After 48 h, frass was visible and all beetles had successfully bored into the challenged plants of *P. indica* and *P. palustris*, whereas no boring activity or frass was visible on the *Q. alba* and mock-inoculated controls (Fig. 1). After 8 weeks, all *X. glabratus*-challenged plants of *P. indica* and *P. palustris* displayed laurel wilt symptoms, while the control plants remained healthy. Typical colonies of *R. lauricola* were isolated from four of the five plants of *P. indica* and the *P. palustris*. Total genomic DNA was extracted from a single spore isolate (PL1529) recovered from a symptomatic *P. indica*, and a partial ribosomal small sub-unit fragment was amplified and sequenced with primers NS1 and NS4 (5). A BLASTn search showed, complete homology with multiple *R. lauricola* GenBank accessions, including RL4 (100%

similarity, and a total score of 1796). The sequence was deposited in GenBank under the accession KC333878.

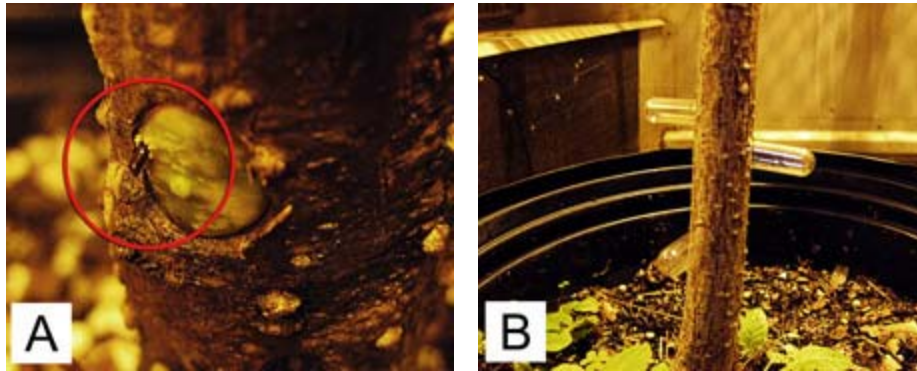


Fig. 1. Growth chamber beetle challenge experiments: (A) *X. glabratus* entering the lower stem of *P. indica*; (B) *P. palustris* mock-inoculated control depicting placement of gelatin capsules onto host.

Persea indica is an ancient member of the Lauraceae, endemic to Macaronesia. This species is a dominant member of the laurel forests (laurisilva) in the Madeira and Canary Islands, and an ornamental in the Mediterranean climates of the USA and Spain (1). The knowledge that *P. indica* wood is attractive to the vector and the above experiments demonstrating pathogenicity indicates that monitoring efforts must consider this species as a potential host and reservoir for the pathogen and vector. The introduction of laurel wilt in areas where *P. indica* is native may prove destructive to the environmentally significant laurisilva forests of Macaronesia. In addition, *P. indica* trees in proximity to the *P. americana* (avocado) production areas of California and Spain may act as a host-bridge, bringing laurel wilt to these areas that have yet to be affected by the disease.

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