



# Population studies of *Raffaelea lauricola* causing laurel wilt disease in the southeastern United States

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## Introduction

Redbay (*Persea borbonia*; Lauraceae), a tree native to the southeastern United States is being devastated by laurel wilt, a disease caused by *Raffaelea lauricola* (T.C. Harr., Fraedrich & Aghayeva sp. nov.) (1) that is vectored by the exotic redbay ambrosia beetle (*Xyleborus glabratus*). The disease is characterized by uniform wilting of the crown with brown to purple leaf discoloration that persists after the tree has died (2). Extensive vascular discoloration also develops in affected trees. Laurel wilt was first observed in 2003 around Savannah, Georgia, and has since spread rapidly through coastal plain forests of southern South Carolina, Georgia and Florida (2). Previous studies have not addressed the genetic diversity and population structure of this pathogen throughout its known range. A preliminary survey of genetic diversity of *R. lauricola* isolates recovered from infected hosts in FL, SC, and GA were analyzed using randomly amplified polymorphic DNA (RAPD).

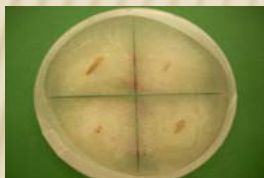


Figure 1. *Raffaelea lauricola* grown on CSMA medium



Figure 2. Redbay infected with laurel wilt disease. Notice the persistent brown leaves after tree death.



Figure 3. Sapwood discoloration of infected Redbay tree. Arrows showing entrance holes of the Redbay ambrosia beetle.

## Methods and Materials

Infected wood samples with sapwood staining were surface disinfested in a 50% (v/v) bleach solution for 30 seconds. The wood chips were then placed on CSMA plates(2), a medium selective for *Ophiostoma* and its related *Raffaelea* anamorph. After 7 days samples were evaluated for fungal growth, and single spore isolations were made from the developing fungus. DNA was extracted as described by Justesen et al. (4) with minor modifications. RAPD-PCR analysis was performed using 10 primers (BB07, AZ14, Y15, Y16, AO13, OPA4, OPA9, OPA12, OPA16, and OPA18) (3). The amplification products were separated by gel electrophoresis on 1.5% agarose gels, stained with SYBR Green. Scoring analysis was done by comparing bands; their presence, absence, and intensity.

## RAPD-PCR Results

The RAPD primers AZ14 and OPA 4 gave reproducible and intense banding patterns. Several isolates had distinct and noticeably different bands, indicating genetic variation among the sampled populations.

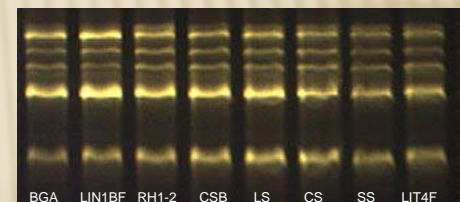


Figure 4. Amplification products of RAPD primer AV08. Banding patterns show no variation. All isolates were collected from Georgia.

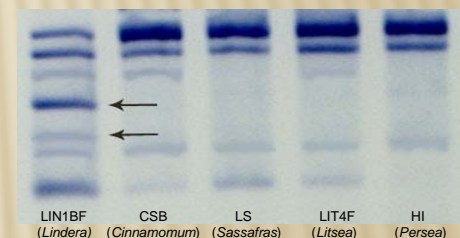


Figure 5. Amplification products of RAPD primer OPA 4. Arrows indicate bands of variation. Lane 1, LIN1BF(*Lindera*) shows genetic heterogeneity among affected genera within the Lauraceae.

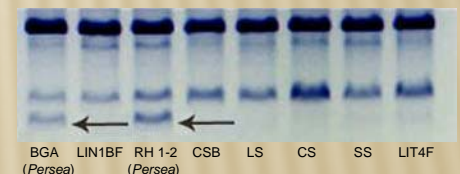


Figure 6. Amplification products of RAPD primer AZ14 among Georgia isolates. Arrows indicate bands of variation for the *Persea* (redbay) isolates against other members of the Lauraceae.

## Discussion

Primers AZ14 and OPA 4 revealed genetic variation among isolates on the same host (redbay, *Persea borbonia*), as well as showing variation between other affected genera within the Lauraceae. The apparent heterogeneity of the *R. lauricola* isolates sampled will lead to further work to assess population structure and disease resistance within the Lauraceae.

Table 1. Origin and host of *R. lauricola* isolates

Isolate	Host	Location
Avocado B	Avocado ( <i>Persea americana</i> )	Jacksonville, FL
Tree 3 Milton	Redbay ( <i>Persea borbonia</i> )	Jacksonville, FL
Tree 4 Milton	Redbay ( <i>Persea borbonia</i> )	Jacksonville, FL
Hawthorne 1	Redbay ( <i>Persea borbonia</i> )	Hawthorne, FL
Hawthorne 4	Redbay ( <i>Persea borbonia</i> )	Hawthorne, FL
Lee, Sass	Sassafras ( <i>Sassafras albidum</i> )	Liberty co., GA
Litsea 4 Eff	Pondspice ( <i>Litsea aestivalis</i> )	Effingham co., GA
Lindera 1B Eff	Pondberry ( <i>Lindera melissifolia</i> )	Effingham co., GA
Pierce co.	Redbay ( <i>Persea borbonia</i> )	Pierce co., GA
CSB	Camphor ( <i>Cinnamomum camphora</i> )	Shellman's Bluff, GA
HI-7 Beetle Raff	Ambrosia Beetle ( <i>Xyleborus glabratus</i> )	Hunting Island, SC
Hunting Island	Redbay ( <i>Persea borbonia</i> )	Hunting Island, SC
Rb1 Bamberg	Redbay ( <i>Persea borbonia</i> )	Bamberg, SC
Ace Basin	Redbay ( <i>Persea borbonia</i> )	Ace Basin Preserve, SC
Edisto Island	Redbay ( <i>Persea borbonia</i> )	Edisto Island, SC

(1) Harrington, T. C., Fraedrich, S.W., and Aghayeva, D.N. 2008. *Raffaelea lauricola*, a new ambrosia beetle symbiotic and pathogen on the Lauraceae. Mycotaxon. 104: 399.

(2) Fraedrich, S. W., Harrington, T. C., Rabaglia, R. J., Ulyshen, M. D., Mayfield, A. E., III, Hanula, J. L., Eickwort, J. M., and Miller, D. R. 2008. A fungal symbiotic of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Dis. 92:215-224.

(3) Solla, A., Dacasa, M.C., Nasmith, C., Hubbes, M., and Gil, L., and 2008. Analysis of Spanish populations of *Ophiostoma ulmi* and *O. novo-ulmi* using phenotypic characteristics and RAPD markers. Plant Pathology. 57: 33-44.

(4) Justesen, A.F.; Ridout, C.J.; Hovmoller, M.S. 2002. The recent history of *Puccinia striiformis* f.sp. tritici in Denmark as revealed by disease incidence and AFLP markers. Plant Pathology. 51: 13-23