Disease biology and host-pathogen interactions

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Hosts

- Lauraceae (Magnoliid complex, Laurales)
Hosts

• **Lauraceae** (Magnoliid complex, Laurales)

• **Avocado** (*Persea americana*)
  o MesoAmerican origins
  o Most important crop in family
  o 4.7 million metric tonnes, 2013
  o Mexico no. 1 producer. CA ($350 million) and FL ($50 million) are most important US producers.
Hosts

- Lauraceae (Magnoliid complex, Laurales)
- Avocado (*Persea americana*)
- Native trees
Susceptible species in the Southeastern USA include several native trees:

- **Sassafras albidum**
- **Persea humilis**
- **Licaria trianda**
- **Persea borbonia**
- **Persea palustris**
- **Ocotea coricea**
- **Lindera melissifolia**
Susceptible species in the Southeastern USA include several native trees, some of which are only moderately susceptible:

- Ocotea coricea
- Persea borbonia
- Persea palustris
- Persea humilis
- Sassafras albidum
- Lindera melissifolia
- Lindera trianda
Hosts

• Lauraceae (Magnoliid complex, Laurales)
• Avocado (*Persea americana*)
• Native trees

Southeastern USA: redbay, swamp bay, silk bay, sasafrass (lancewood, gulf licaria)

Tropical America: Lauraceae well represented. Besides avocado, *Persea liebmannii* (aka *podadenia*) and others??
Hosts

- Lauraceae (Magnoliid complex, Laurales)
- Avocado (*Persea americana*)
- Native trees
  
  Southeastern USA: redbay, swamp bay, silk bay, sasafrass, et al.

  Tropical America: Lauraceae well represented. *Persea liebmannii* (aka *podadenia*)

  Asia: Lauraceae also well represented. Hosts?
Pathogen

*Raffaelea lauricola* (Eukaryota: Eumycota: Ascomycota: Ophiostomatales)
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*Raffaelea* spp. are confused taxonomically. They are nutritional symbionts of ambrosia beetles, and are predominantly **not** plant pathogens.
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Diagnosis: Symptoms,
Mayfield

Kendra et al.

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Pathogen

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Diagnosis: Symptoms, **cultural morphology (+ cyclohexamide insensitivity)**,
Pathogen

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Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU)
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Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU) DNA sequences for small subunit (SSU) and large subunit (LSU) of ribosomal RNA have been used to identify *R. lauricola*. However, neither are specific for the pathogen.
For example, a nonpathogenic close relative of *R. lauricola* (PL1004) is also detected with SSU and LSU diagnostics.
Pathogen

*Raffaelea lauricola* (Eukaryota: Eumycota: Ascomycota: Ophiostomatales)

*Raffaelea* spp. are confused taxonomically. They are nutritional symbionts of ambrosia beetles, and are predominantly not plant pathogens.

Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU, SSRs) of the pathogen
Development of Multilocus PCR Assays for *Raffaelea lauricola*, Causal Agent of Laurel Wilt Disease

Tyler J. Dreaden and John M. Davis, School of Forest Resources and Conservation, University of Florida, Gainesville 32611; Carrie L. Harmon, Department of Plant Pathology, University of Florida, Gainesville 32611; Randy C. Ploetz and Aaron J. Palmateer, Tropical Research and Education Center, University of Florida, Homestead 33031; Pamela S. Soltis, Florida Museum of Natural History, and Jason A. Smith, School of Forest Resources and Conservation, University of Florida, Gainesville

Abstract


Laurel wilt, caused by the fungus *Raffaelea lauricola*, is an exotic disease that affects members of the Lauraceae plant family in the southeastern United States. The disease is spreading rapidly in native forests and is now found in commercial avocado groves in south Florida, where an accurate diagnostic method would improve disease management. A polymerase chain reaction (PCR) method based on amplifying the ribosomal small-subunit DNA, with a detection limit of 0.0001 ng, was found to be suitable for some quantitative PCR applications; however, it was not taxon specific. Genomic sequencing of *R. lauricola* was used to identify and develop primers to amplify two taxon-specific simple-sequence repeat (SSR) loci, which did not amplify from related taxa or host DNA. The new SSR loci PCR assay has a detection limit of 0.1 ng of *R. lauricola* DNA, is compatible with traditional and real-time PCR, was tested in four labs to confirm consistency, and reduces diagnostic time from 1 week to 1 day. Our work illustrates pitfalls to designing taxon-specific assays for new pathogens and that undescribed fungi can limit specificity.

Dreaden et al. 2014 identified *R. lauricola*-specific simple-sequence repeats (SSRs)
**Pathogen**

*Raffaelea lauricola* (Eukaryota: Eumycota: Ascomycota: Ophiostomatales)

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**Diagnosis:** Symptoms, cultural morphology, and molecular identity (SSU, LSU, SSRs) of the pathogen

Infection, colonization and host response
These are extremely sensitive pathosystems

Swampbay, *Persea palustris*
These are extremely sensitive pathosystems

Avocado, *Persea americana*
Tylose formation plays a key role in xylem dysfunction
*Raffaelea lauricola* induces tylose formation in infected avocado trees, but is scarcely evident, microscopically. Ten days after inoculation, a gfp-marked strain of the pathogen is: (C) not visible, until (D) 3 days after a bioenrichment step.
Impact of laurel wilt on xylem function

Functional xylem, acid fuchsin assay

dai = days after inoculation. External, internal disease severity: 1 = no symptoms, 10 = dead or 100% symptomatic. % = functional relative xylem, based on acid fuchsin stain/uptake. Scale = 0.5 cm.
Point ▼ indicates inoculation location.
*Raffaelea lauricola* induces tylose formation in infected avocado trees, but is scarcely evident, microscopically. Ten days after inoculation, a gfp-marked strain of the pathogen is: (C) not visible, until (D) 3 days after a bioenrichment step. (E) Vessels that are occluded with tyloses increase soon after inoculation, and (F) xylem dysfunction and reduced hydraulic conductivity are related.
By 30 dai (significant disease), proportions of lumina that are colonized with the pathogen are far lower (less than 1%) than those that are occluded with tyloses (up to 70%).
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How is this possible?
**Pathogen**

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**Infection, colonization and host response**

**Epidemiology**
Anthropogenic dissemination
Anthropogenic dissemination

Redbay Ambrosia Beetle

Firewood Alert
Don't buy bundles of bad bugs!

Don't move firewood

Help stop invasive pests

Buy it where you burn it.
dontmovefirewood.org
Anthropogenic dissemination

Firewood

Woodturners

Redbay bowl with patched ambrosia beetle galleries
Anthropogenic dissemination

Firewood

Wood turners

Barbeque fuel
Anthropogenic dissemination

Firewood

Wood turners

Barbeque fuel

Chipped wood/mulch
Natural dissemination

Laurel Wilt
Suggested Disease Cycle
Fungal Pathogen: Raffaelea lauricola
Insect Vector: Xyleborus glabratus
(redbay ambrosia beetle)

Female beetles emerge and search for host trees

Beetle deposits fungal spores into sapwood during initial attempt to colonize the host

Healthy Redbay

Beetle with spore bearing mycangia

Beetle gallery with fungal symbiont

Extensive black/brown streaking in sapwood

Mild vascular discoloration

Complete Crown Wilt/ Death

Frass tubes (Ephemeral)

Beetles attracted to dead or dying Redbay trees

Partial Crown Wilt

M. Hughes, J. Thomas, and A.E. Mayfield

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Last Revised 9/21/09
Explosive spread occurs in avocado orchards due to rootgraft movement.
Focal spread of laurel wilt on avocado (without sanitation or fungicide protection)

In 9 months, total losses in four disease foci increased by 130 trees (3.6 trees focus$^{-1}$ month$^{-1}$)
Pathogen

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Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU, SSRs) of the pathogen

Infection, colonization and host response

Epidemiology

Alternative vectors?
To date, *R. lauricola* has been detected in at least nine other ambrosia beetle species, seven of which are shown here.
### Recovery of *Raffaelea lauricola* from ambrosia beetles reared from bolts of laurel wilt-affected host trees

<table>
<thead>
<tr>
<th>Species</th>
<th>Reared from laurel wilt-affected bolts of swamp bay, <em>Persea palustris</em></th>
<th>Reared from laurel wilt-affected bolts of avocado, <em>Persea americana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>No. w <em>R. lauricola</em></td>
</tr>
<tr>
<td><em>Xyleborus glabratus</em></td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td><em>Xyleborus affinis</em></td>
<td>41</td>
<td>5</td>
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<tr>
<td><em>Xyleborus bispinatus</em></td>
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<td><em>Xyleborus ferrugineus</em></td>
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<td><em>Xyleborus volvulus</em></td>
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<td><em>Xyleborinus gracilis</em></td>
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<td>26</td>
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<td><em>Xyleborinus saxeseni</em></td>
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<td><em>Xylo. crassiusculus</em></td>
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<td>1</td>
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<td><em>Ambro. develexulus</em></td>
<td>25</td>
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<tr>
<td><em>Ambro. lecontei</em></td>
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<td>0</td>
</tr>
<tr>
<td><em>Hypothenemus sp.</em></td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Totals/means</strong></td>
<td>473</td>
<td>35.3</td>
</tr>
</tbody>
</table>

* From 75 bolts of laurel wilt-affected avocado
Summary

- Laurel wilt host range generally restricted to Lauraceae plant family. New world taxa are most susceptible
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• *Raffaelea lauricola* is the only systemic and lethal pathogen in a genus of predominantly nonpathogenic ambrosia beetle symbionts.

• Diagnosis difficult for such a poorly known and understood group of fungi.
Summary

• Laurel wilt host range generally restricted to Lauraceae plant family. New world taxa are most susceptible

• *Raffaelea lauricola* is the only systemic and lethal pathogen in a genus of predominantly nonpathogenic ambrosia beetle symbionts

• Diagnosis difficult for such a poorly known and understood group of fungi

• Susceptible hosts are extremely sensitive to *Raffaelea lauricola* – 100 conidia lethal. Symptom induction is a mystery
Summary

- Spread occurs by natural and unnatural means.
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  - Natural spread
    - ambrosia beetles – *Xyleborus glabratrus*
      for SE USA natives and ? for avocado
Summary

- Spread occurs by natural and unnatural means.
- Natural spread
  - ambrosia beetles – *Xyleborus glabratus* for SE USA natives and ? for avocado
- Unnatural (anthropogenic) spread is responsible for the biggest geographic jumps in distribution
Laurel Wilt Disease is a destructive disease of red bay (Persea borbonia), and other species within the laurel family (Lauraceae) caused by a vascular wilt fungus (Raffaelea lauricola) that is vectored by the red bay ambrosia beetle (Xyleborus glabratus). The pathogen has been confirmed through laboratory analysis of host samples collected in the counties highlighted.

Initial Detection of Xyleborus glabratus - May 2002
Port Wentworth, GA

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Gulf of Mexico

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Special thanks to:

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