Towards the Development of a Laurel Wilt Screening Program in Redbay
(Persea borbonia)

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University of Florida
Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry
RAB- *Xyleborus glabratus*

Photographs by: Lyle J. Buss, University of Florida
Mycangia → Budding yeast
Media → Hyphal

1\textsuperscript{st} known ambrosia beetle symbiont to cause a vascular wilt

Most abundant/frequent of six \textit{Raffaelea} spp. isolated from mycangia

Females have been estimated to carry up to 30,000 CFU in mycangia

Photograph by: Harrington et al. 2008
Redbay- *Persea borbonia*
Objective

1. Develop a protocol for locating and screening putatively resistant redbays
   - Identification of candidates in the field
   - Disease pressure survey
   - Vegetative propagation
   - Pathogen genetic diversity
   - Disease screening
Identification of Candidate Germplasm

Six field locations
Established disease centers
High mortality rate (> 90%)

Image: Koch and Smith 2008
## Current Status

Sites revisited in early 2011

**Survivorship: 61/83 ~ 73%**

<table>
<thead>
<tr>
<th>Site</th>
<th>Count</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fort George Island, FL</td>
<td>10/10</td>
<td>2004</td>
</tr>
<tr>
<td>Fort Clinch, FL</td>
<td>8/22</td>
<td>2006</td>
</tr>
<tr>
<td>Cumberland Island, GA</td>
<td>8/10</td>
<td>2005</td>
</tr>
<tr>
<td>St. Catherines Island, GA</td>
<td>21/21</td>
<td>2005</td>
</tr>
<tr>
<td>Hunting Island, SC</td>
<td>8/10</td>
<td>2004</td>
</tr>
<tr>
<td>Edisto Island, SC</td>
<td>6/10</td>
<td>2004</td>
</tr>
</tbody>
</table>
Redbay Rooting Experiments

1. Effects of IBA and Bottom Heat
2. The Influence of Rooting Media
Results: IBA and Bottom Heat

No significant differences between treatments
Results: Rooting Media

Disease outbreak:
- Transplant damping off
- Severe mortality in bench
- Suspected *Cylindrocladium* and *Fusarium* spp.
Pathogen Diversity

Single introduction event ≈ Little genetic diversity
Multiple introductions ≈ Increased genetic diversity

**AFLP**: Amplified Fragment Length Polymorphism

Fifty-six isolates from 8 hosts
Six primer pairs + 3 selective nucleotides
GeneMarker V1.70
### Distributions

<table>
<thead>
<tr>
<th></th>
<th>FL</th>
<th>GA</th>
<th>SC</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. borbonia</em></td>
<td>21</td>
<td>10</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td><em>P. palustris</em></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>P. americana</em></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. albidum</em></td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. aestivalis</em></td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. melissifolia</em></td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C. camphora</em></td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>X. glabratus</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

**Host Plant**
- ● *P. borbonia*
- ○ *P. americana*
- ■ *S. albidum*
- □ *C. camphora*
- ◆ *P. palustris + L. aestivalis*
- ◇ *P. borbonia + X. glabratus*
- ☀ *L. melissifolia + L. aestivalis*
- ▲ *P. borbonia + P. americana + P. palustris*
- ▲ *P. borbonia + C. camphora*
Pathogen Diversity Results

Over 218 fragments generated per sample
Fifty-three of the 56 isolates identical
Three isolates polymorphic at 1-2 loci

Polymorphic Isolates: → Pathogenicity Tests
↘ Sequencing rDNA
AFLP- Conclusion

• All isolates are nearly identical (99% similarity)
• Suggests *R. lauricola* is a clonal population that entered in a single introduction event
• No evidence of pathogenic variability – screening will not require diverse pathogen population for inoculum
• **SSRs now available for future monitoring**
Preliminary Disease Screening: Experimental Design

Ten clones
Four replicates/clone
Field susceptible control
Complete randomized block
Plant Science and Education Unit
Citra, Florida
Establishment period of 8 weeks
Inoculations

Two drill wounds on lower stem
50 μl of spore suspension per wound
1.0 x 10^6 spores/mL – MK1 isolate
Similar to DED protocols
Rating Scale

0= no wilt symptoms

1= less than or equal to 1/3 of the crown wilted

2= greater than 1/3, but less than or equal to 2/3 of crown wilted

3= greater than 2/3 of crown wilted, but tree still has some green, turgid foliage

4= tree dead, completely wilted
Results/ Observations

- Wilt symptoms developed within 3 weeks of inoculation
- Clone FGC displayed a significant difference in disease ratings when compared to other genotypes
- Clone FGC is currently alive in the field
- Some genotypes displayed a re-flushing of the canopy, after the development of wilt symptoms (freezes killed)
Field Trial #2

Fifty Clones (current field survivors)
FGC replicated 6x in plot
Inoculations in Spring 2012
Inoculum Threshold

Is 100,000 spores a realistic concentration?
What is the minimum CFU level to produce symptoms?
Under lower CFU conc. is tree recovery possible?

<table>
<thead>
<tr>
<th>CFU Concentration</th>
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<tbody>
<tr>
<td>100,000</td>
</tr>
<tr>
<td>10,000</td>
</tr>
<tr>
<td>1,000</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>0</td>
</tr>
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</table>

“Thousands of spores of *R. lauricola* may ooze from the mycangia and infect the severed vessels, resulting in systemic colonization” Harrington and Fraedrich 2010
Acknowledgements

Funding from USDA-Forest Service, R8
Dr. Jason Smith (Major Advisor, UF)
Dr. Albert Mayfield III (NC DOF)
Dr. Randy Ploetz (UF)
Dr. Pamela Soltis(UF)
Dr. Ariena van Bruggen(UF)

Dr. Claire Anderson
Kathy Smith
Samuel Glucksman
Keumchul Shin
Candace Palmer
Kathy Slifer
Steve Fraedrich (AFLP isolates)
Forest pathology lab
Questions?