Tolerance in *Persea borbonia* and Future Restoration

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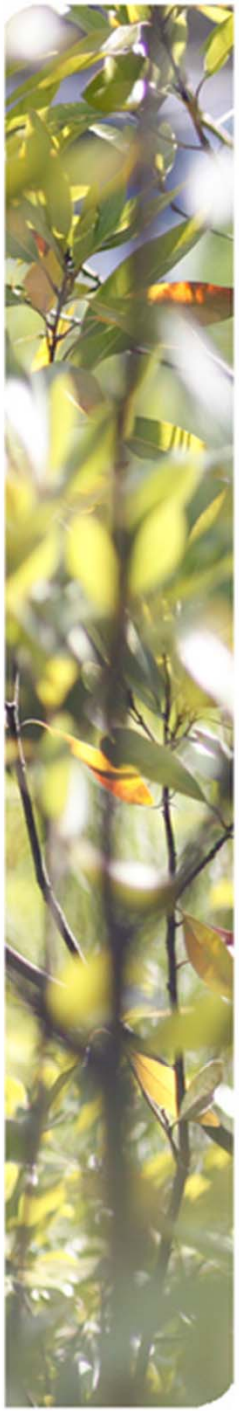
Laurel Wilt Resistance Screening Program

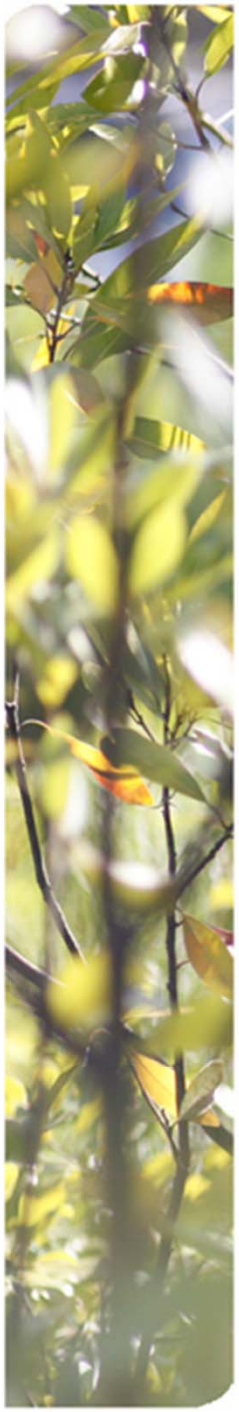
Objectives:

- **Identify & propagate survivor redbays**
- **Assess genetic diversity of *R. lauricola***
- **Optimize inoculum dosage**
- **Screen for resistance and/or tolerance**
- **Future projects**



Published Mortality Rates

- **90%** on Ft. George Island, FL (Fraedrich et al. 2008)
- **100%** of over-story layer in Etoniah Creek, FL (Shields et al. 2011)
- **98%** (2004-2009) on St. Catherines Island, GA (Evans et al. 2013)
- **90%** (over 3 cm DBH) in GA (Spiegel and Leege 2013)



Identification of Survivors

- **Sampling occurred 2009 – 2013**
  After 1st wave of dieback
- **Sites with high mortality**
  FL (Ft. Clinch & Ft. George Island)
  GA (Cumberland & St. Catherines Is.)
  SC (Hunting & Edisto Island)
- **Asymptomatic survivors**
  Over 7.5 cm dbh
  Stem cuttings f/ 94 trees
  GPS labeled
  Monitored yearly

Propagation of Survivors

What was known

- Seed is the standard
- Lauraceae difficult to root
- Deghan & Sheehan 1991

Rooting Experiments

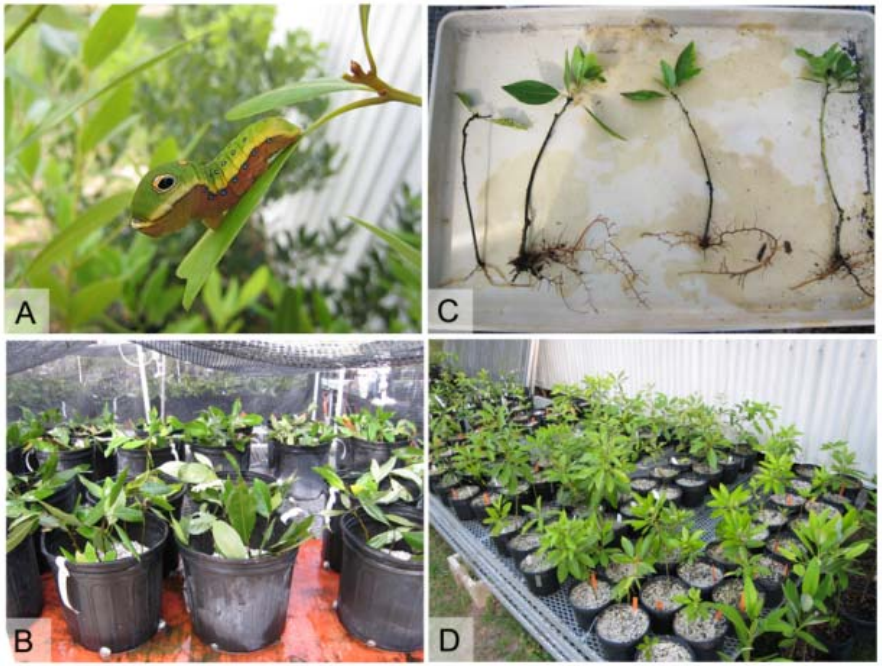
- 1: IBA conc. x Bottom Heat
- 2: Rooting Media

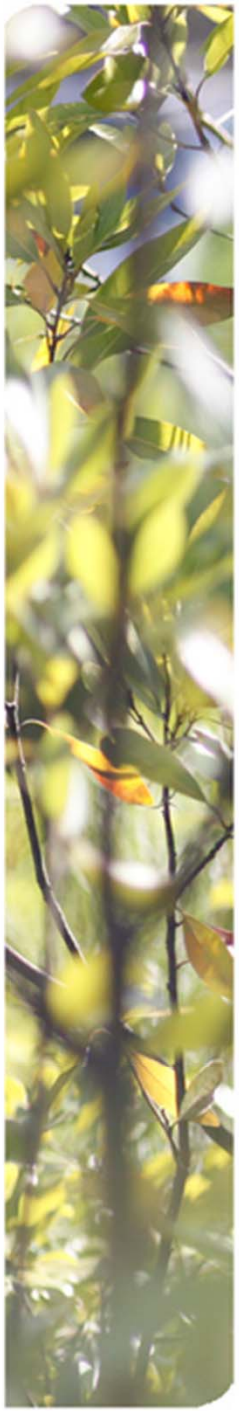
REFEREED RESEARCH

Vegetative propagation of putatively laurel wilt-resistant redbay (*Persea borbonia*)

Native Plants Journal 2014, vol. 15, no. 1

Marc A Hughes and Jason A Smith





Propagation of Survivors

Exp. 1: IBA conc. x Bottom Heat

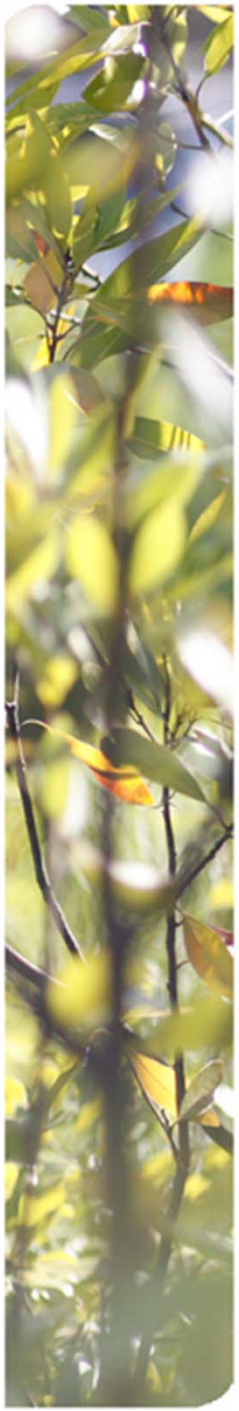
Experiment 1: P values for provenance, IBA level, and presence or absence of heat and their interactions on rooting response of redbay cuttings.

Source of variation	df[z]	Rooting (%)	Rooting of live cuttings only (%)	Roots per cutting (mean)	Longest root per cutting (mean in cm)
Provenance (P)	5	0.193	0.559	0.225	0.002
IBA (I)	1	0.515	0.084	0.078	0.181
Heat (H)	1	0.457	0.932	0.563	0.492
H x I	1	0.548	0.718	0.693	0.857
H x P	5	0.019	0.010	0.164	0.211
P x I	5	0.412	0.113	0.720	0.852
H x P x I	5	0.584	0.731	0.879	0.709

[z] df = degrees of freedom; $n = 54$; number of observations = 216.

Mean value: 24-27% 60-68% 2.7-3.6 11.3-13.3

Effect of clone most significant (P = 0.0001)



Propagation of Survivors

Exp. 2: Effect of Rooting Media

TABLE 2

Experiment 2: The rooting responses (mean ± standard error) and P values of redbay cuttings in 4 rooting media.

Media	Key	Rooting (%)	Rooting of live cuttings only (%)	Roots per cutting (mean)	Longest root per cutting (mean in cm)
3:1 P:V	1	37.0 ± 6.8 a	71.6 ± 8.4 a	3.1 ± 0.2 a	11.8 ± 1.9 a
1:1:1 P:PT:SD	2	19.5 ± 4.8 b	48.7 ± 10.6 bc	2.0 ± 0.2 a	12.1 ± 1.9 a
1:1 P:PT	3	28.9 ± 6.1 ab	66.8 ± 9.3 ab	1.7 ± 0.2 a	15.2 ± 1.9 a
2:1:1 P:SD:S	4	20.0 ± 4.9 b	46.4 ± 10.6 c	2.2 ± 0.2 a	11.7 ± 1.9 a

1. 3:1 (Perlite:Vermiculite) -----Best Rooting Medium
2. 1:1:1 (Perlite:Peat:Sawdust)
3. 1:1 (Perlite:Peat)
4. 2:1:1 (Peat:Sawdust:Sand)

Effect of clone most significant (P = 0.0001)

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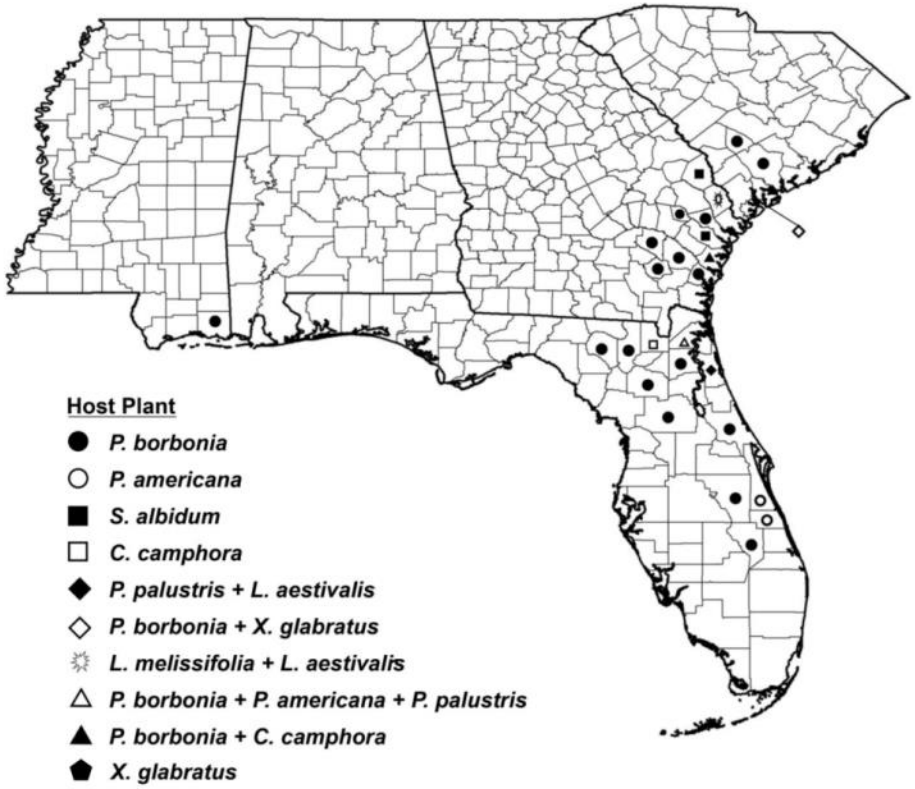
Assess Genetic Diversity of *R. lauricola*



Samples

- 57 isolates across SE USA
- 2004-2010 (founder population)
- 8 hosts

AFLP Markers

- 218 fragments/isolate

Results

- 54/57 isolates identical
- Single dominant clone in USA
- Suggests 1 introduction event
- Similar virulence

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Inoculation Optimization

Forest Pathology WILEY-BLACKWELL

For. Path.
doi: 10.1111/efp.12134
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Responses of swamp bay, *Persea palustris*, and avocado, *Persea americana*, to various concentrations of the laurel wilt pathogen, *Raffaelea lauricola*

By M. A. Hughes[1,5], S. A. Inch[2], R. C. Ploetz[3], H. L. Er[4], A. H. C. van Bruggen[4] and J. A. Smith[1]

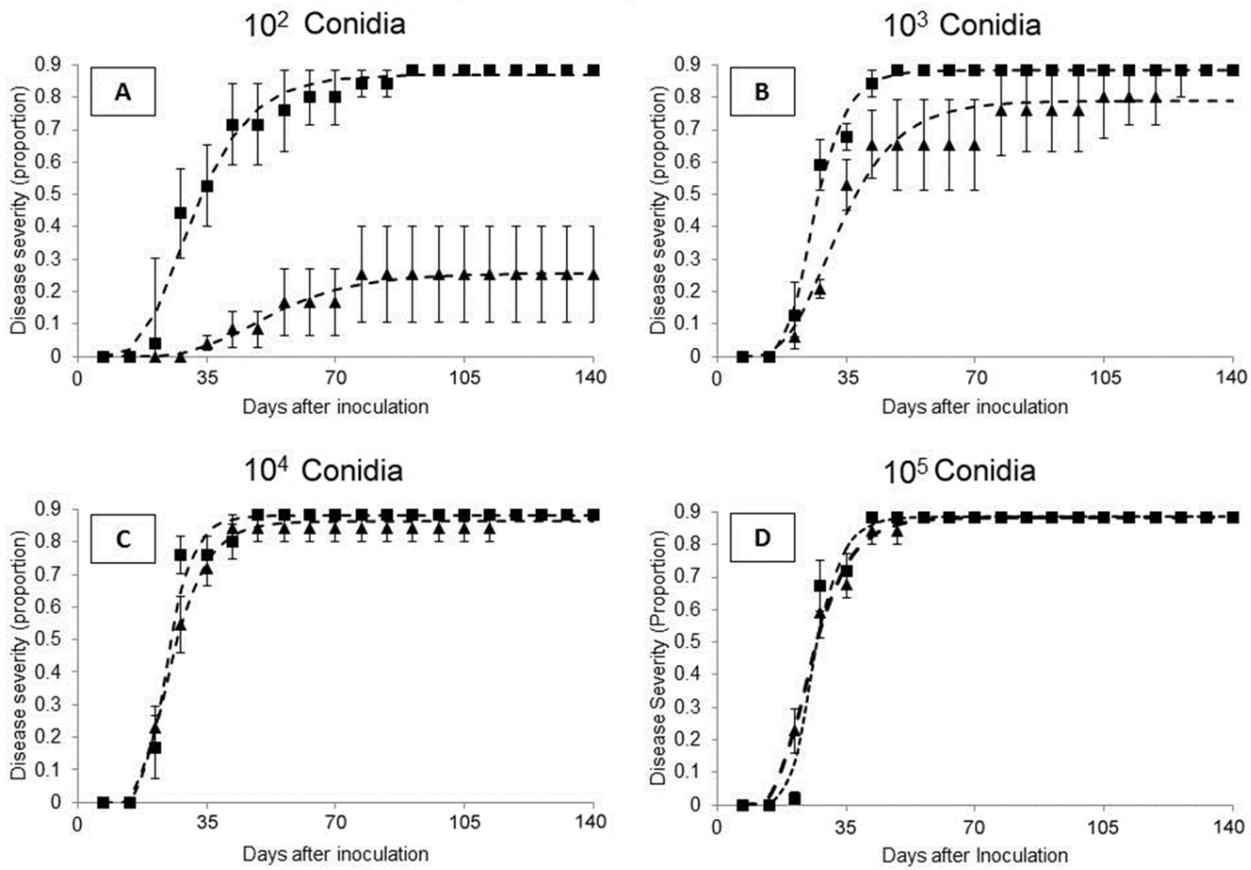
Objectives

1. **Assess if symptom development is dependent on pathogen conc.**
2. **Determine lower threshold of *R. lauricola* needed to incite LW**
3. **Determine if *R. lauricola* can migrate into swamp bay roots**

Treatments

- **Potted plants (1 – 1.5 m tall)**
- **Conidia concentration: 10^2 – 10^5**
- **Location: outdoors vs. greenhouse**

Disease progress curves for swamp bay



Hughes et al. 2015

Results

- 10^{3-5} conidia = similar symptom development (internal and external)
- 10^{2} conidia = lower threshold, environment dependent
- *R. lauricola* isolated f/ swamp bay roots (77% of symptomatic plants)
- Avocado: 10^{2-3} had less symptoms than 10^{4-5} conidia/plant

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Resistance Screening Trials

Field plot, pre-inoculation, 2014

Inoculation Trials

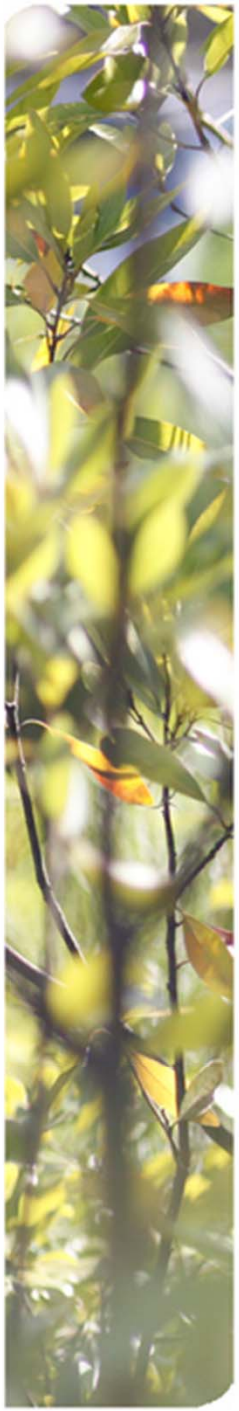
- **Vegetatively propagated plants (PSREU – Citra FL)**
- **Aqueous spore suspension (PL571 - USA dominant isolate)**
- **Water inoculated controls**

3 field trials

- **2010: Pilot study (10 genotypes)**
- **2012: Experiment 1 (18 genotypes)**
- **2014: Experiment 2 (31 genotypes)**

Disease Parameters

- **Incubation period, Rate, Wilt severity, AUDPC, Mortality**



Experiment 1 (2010)

- **Pilot Study** (10 tree genotypes tested, 4 replicates)
- 10^5 conidia per tree

Results

Clone	Incubation (d)	Rate	AUDPC	Final Severity %	Mortality %
FGC	14.0 a	0.26 a	39.51 ± 7.7 a	56.8. ± 6.0 b	50 a
All Others	14.0–15.8 a	0.11–0.31 a	54.3–60.8 ab, 70.4–73.1 b	88.5 ± 6.0 a	100 b

Conclusions

- 10^5 conidia may be too much
- Dosage should be relevant to vector
- FGC has tolerance, even at this extreme dosage level

Experiment 2 (2012)

- **18 tree genotypes (4-6 replicates per tree)**
- **3.0×10^3 conidia/tree (based on Hughes et al. 2015 and vector capacity)**

<u>Results</u>

- **3 clones (FGC, HIA and HIL)**
- **Lower: incubation, rate, AUDPC and Disease severity**
- **No mortality among clones**
- **Trees showed a consistent tolerance among replicates**

Experiment 3 (2014)

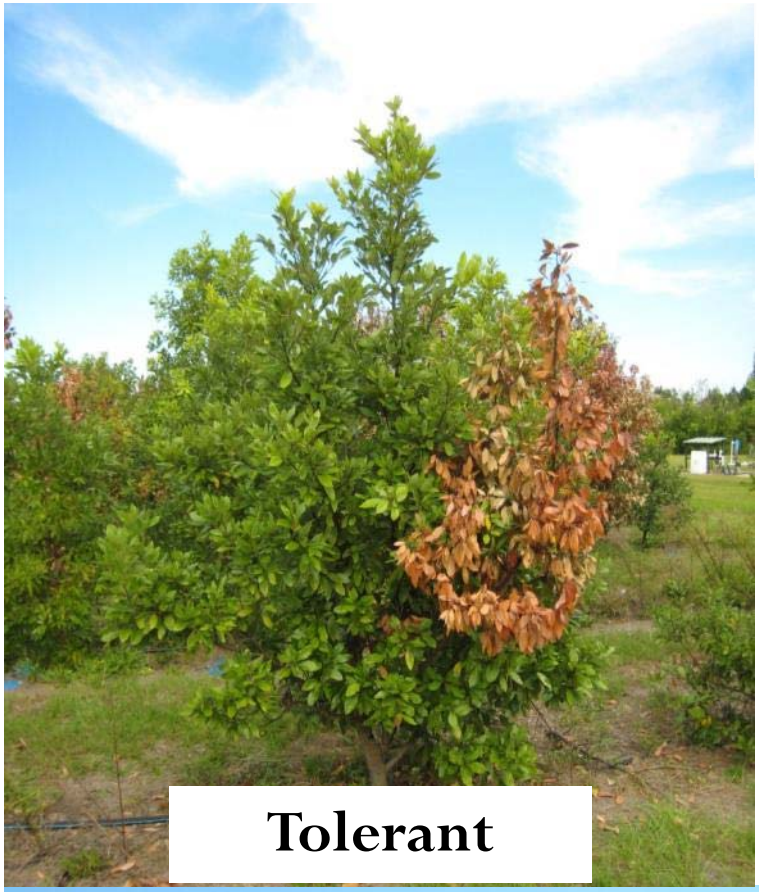
- **31 tree genotypes** **(4-6 replicates per tree)**
- **3.0 x 10^3 conidia/tree** **(based on Hughes et al. 2015 and vector capacity)**
- **Re-inoculate living replicates from Exp. 2**

<u>Results</u>

- **7 tolerant clones (FGC, HIA ,HIL + FGJ, CIH, FCP, CIG)**
- **Lower: Rate, AUDPC and Disease severity**
- **No mortality among clones**
- **Trees showed a consistent tolerance among replicates**
- **Re-inoculation had no effect on disease development**

2014 Trial

12 weeks after inoculation



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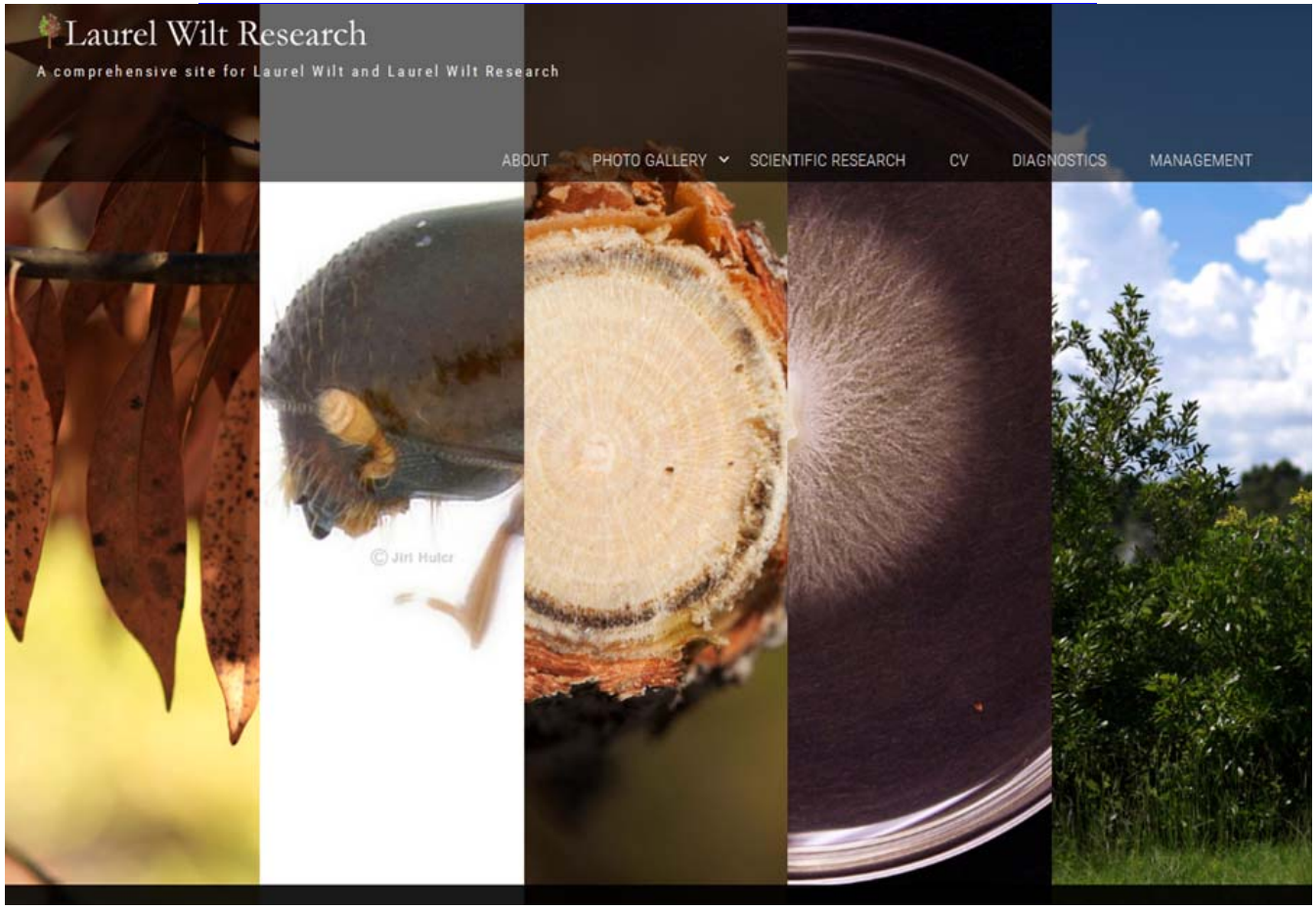
Future Projects

- Screen more genotypes for resistance
- Assess horticultural traits of tolerant redbays
- Genotype tolerant redbays and seedling progeny to find useful bio-markers

  Collaboration w/ K. Smith & J. Davis (UF Gainesville)

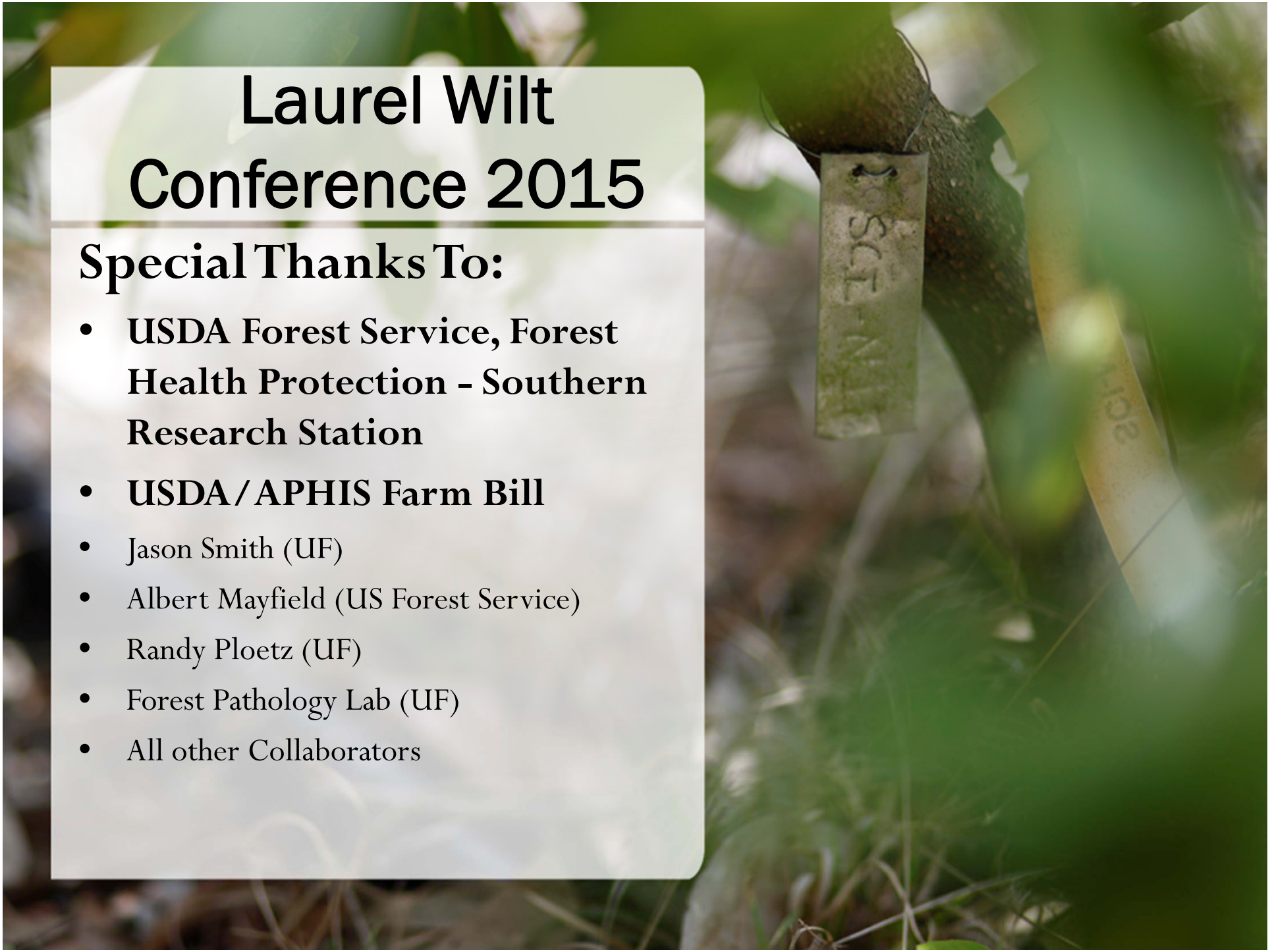
Laurel Wilt Research

www.laurelwiltresearch.com



-Site is under development -Contact me for info to add content (mhughes741@ufl.edu)

Laurel Wilt Conference 2015

Special Thanks To:

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