Disease biology and host-pathogen interactions

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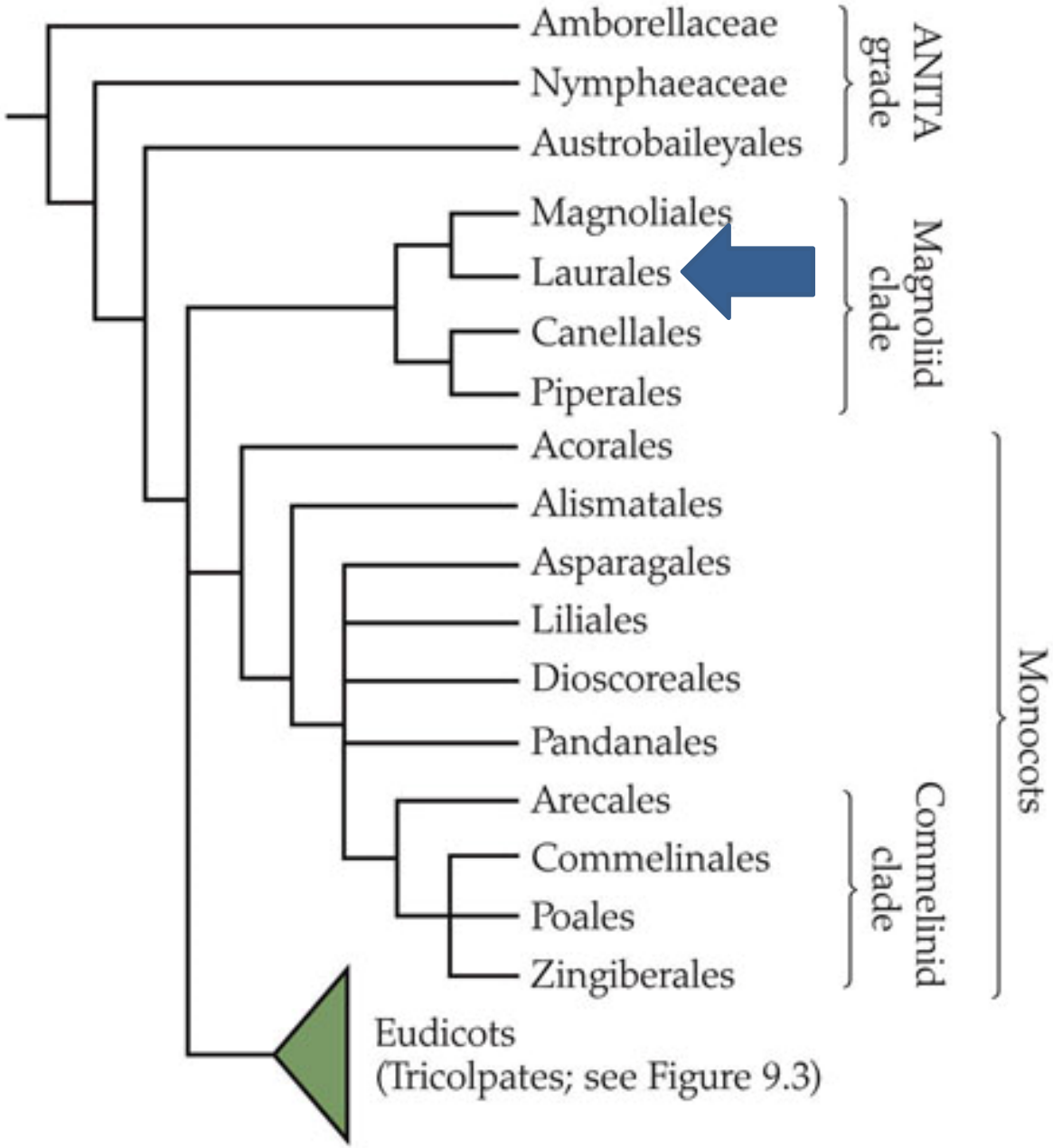
Conference on Laurel Wilt Disease and Natural Ecosytems:
Impacts, Mitigation and the Future
June 16-18, 2015 | Coral Springs, Florida

UF IFAS Research
UNIVERSITY of FLORIDA

Hosts

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



***PLANT SYSTEMATICS, Third Edition*, Figure 9.2**

Hosts

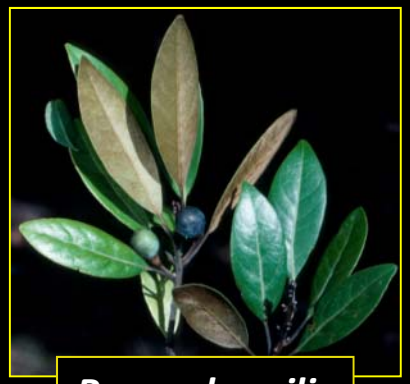
- Lauraceae (Magnoliid complex, Laurales)
- Avocado (*Persea americana*)
  - MesoAmerican origins
  - Most important crop in family
  - 4.7 million metric tonnes, 2013
  - Mexico no. 1 producer. CA ($350 million) and FL ($50 million) are most important US producers.

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

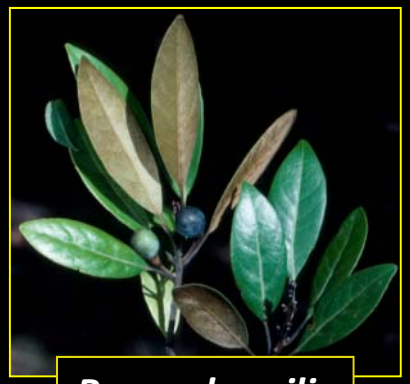
Persea palustris

Ocotea coricea

Lindera melissifolia

Susceptible species in the Southeastern USA include several native trees, **some of which are only moderately susceptible**

Sassafras albidum



Persea humilis

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Lindera melissifolia

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?

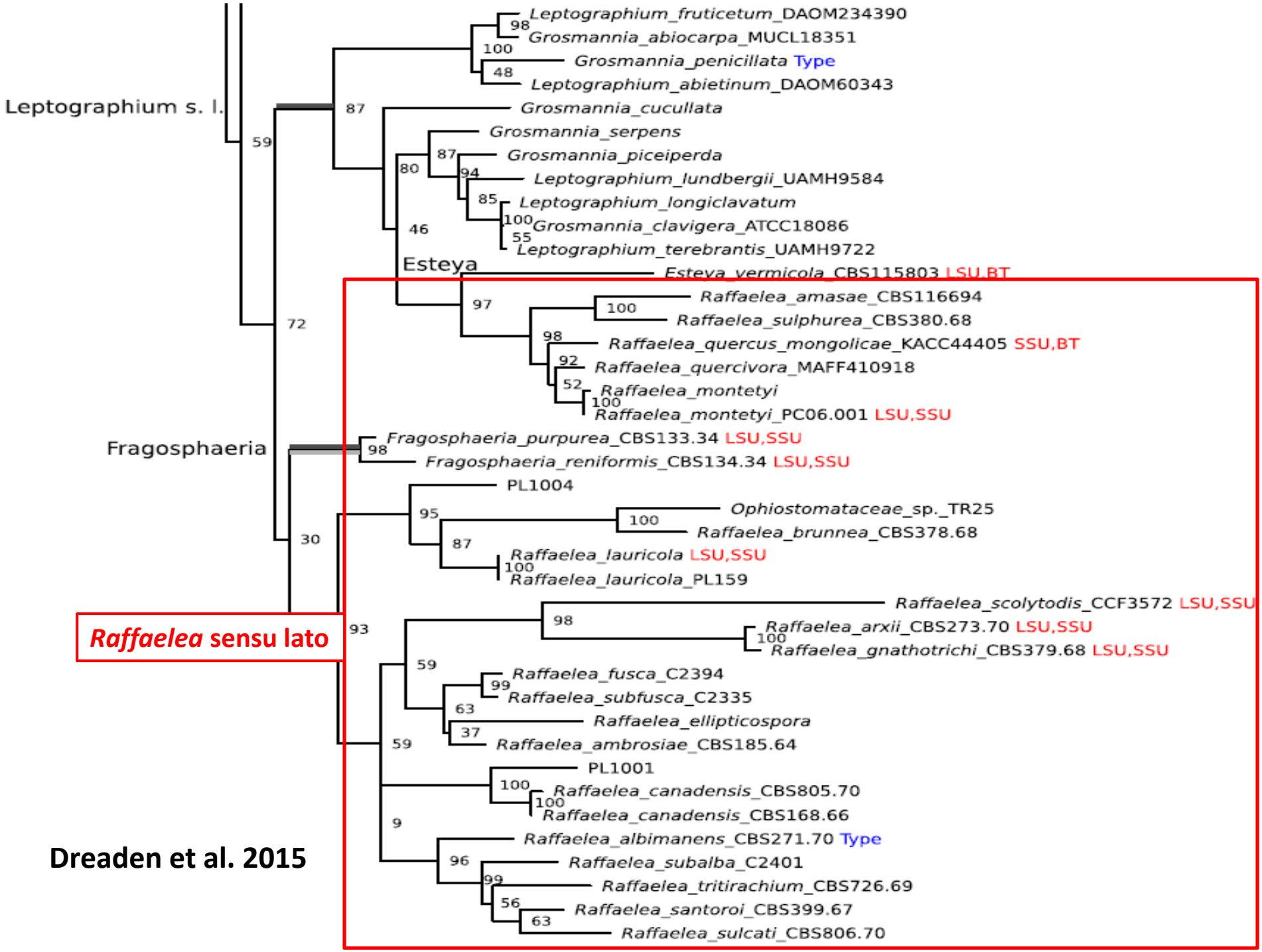
Pathogen

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

Pathogen

Raffaelea lauricola (Eukaryota: Eumycota: Ascomycota: Ophiostomatales)

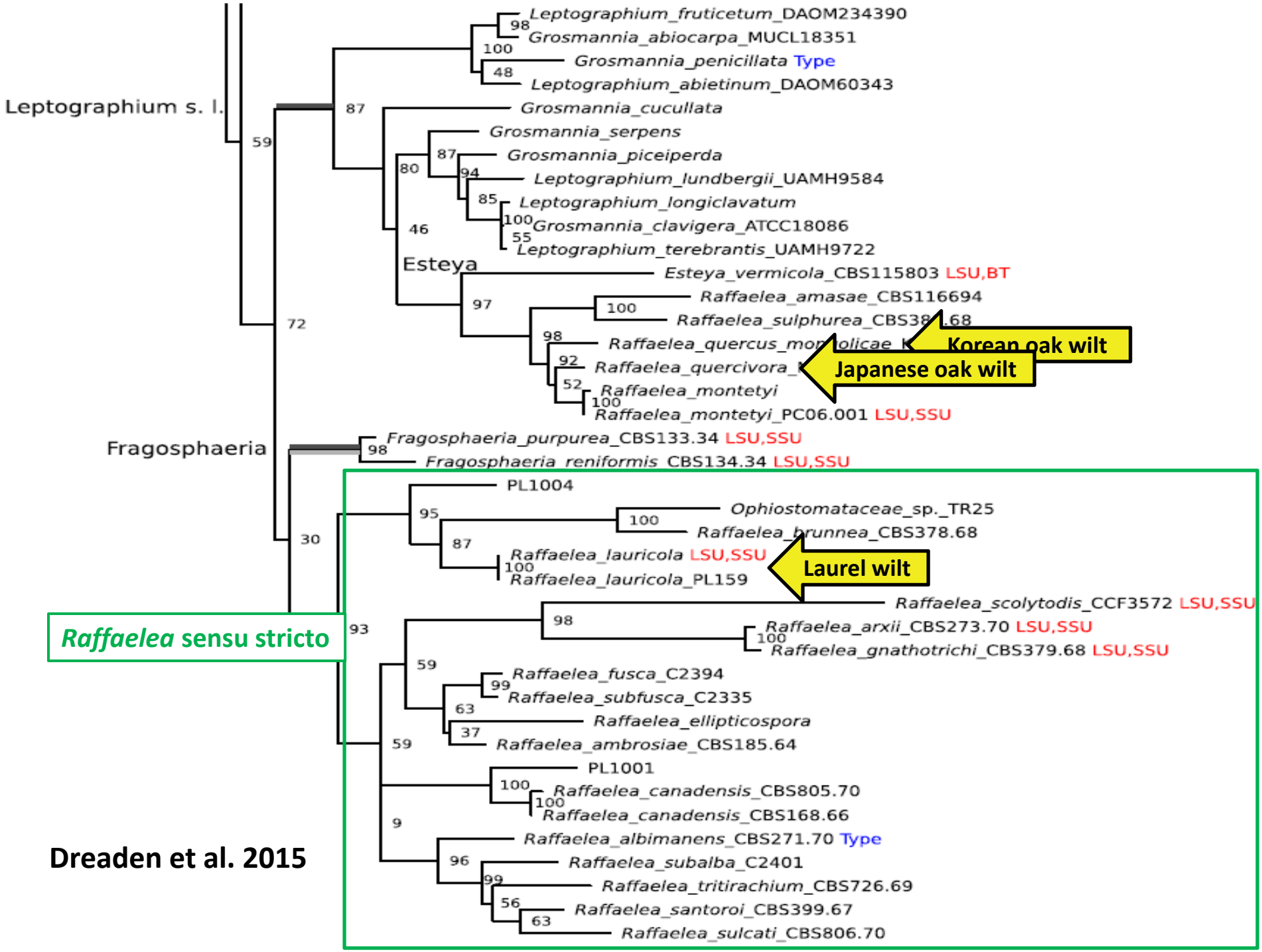
***Raffaelea* spp. are confused taxonomically.**


Leptographium s. l.
Leptographium_fruticetum_DAOM234390
Grosmannia_abiocarpa_MUCL18351
Grosmannia_penicillata Type
Leptographium_abietinum_DAOM60343
Grosmannia_cucullata
Grosmannia_serpens
Grosmannia_piceiperda
Leptographium_lundbergii_UAMH9584
Leptographium_longiclavatum
Grosmannia_clavigera_ATCC18086
Leptographium_terebrantis_UAMH9722
Esteya
Esteya_vermicola_CBS115803 LSU,BT
Raffaelea_amasae_CBS116694
Raffaelea_sulphurea_CBS380.68
Raffaelea_quercus_mongolicae_KACC44405 SSU,BT
Raffaelea_quercivora_MAFF410918
Raffaelea_montetyi
Raffaelea_montetyi_PC06.001 LSU,SSU
Fragosphaeria
Fragosphaeria_purpurea_CBS133.34 LSU,SSU
Fragosphaeria_reniformis_CBS134.34 LSU,SSU
PL1004
Ophiostomataceae_sp._TR25
Raffaelea_brunnea_CBS378.68
Raffaelea_lauricola LSU,SSU
Raffaelea_lauricola_PL159
Raffaelea_scolytodis_CCF3572 LSU,SSU
Raffaelea_arxii_CBS273.70 LSU,SSU
Raffaelea_gnathotrichi_CBS379.68 LSU,SSU
Raffaelea_fusca_C2394
Raffaelea_subfusca_C2335
Raffaelea_ellipticospora
Raffaelea_ambrosiae_CBS185.64
PL1001
Raffaelea_canadensis_CBS805.70
Raffaelea_canadensis_CBS168.66
Raffaelea_albimanens_CBS271.70 Type
Raffaelea_subalba_C2401
Raffaelea_tritirachium_CBS726.69
Raffaelea_santoroi_CBS399.67
Raffaelea_sulcati_CBS806.70
Raffaelea sensu lato
Dreaden et al. 2015

Pathogen

Raffaelea lauricola (Eukaryota: Eumycota: Ascomycota: Ophiostomatales)

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



Dreaden et al. 2015

Pathogen

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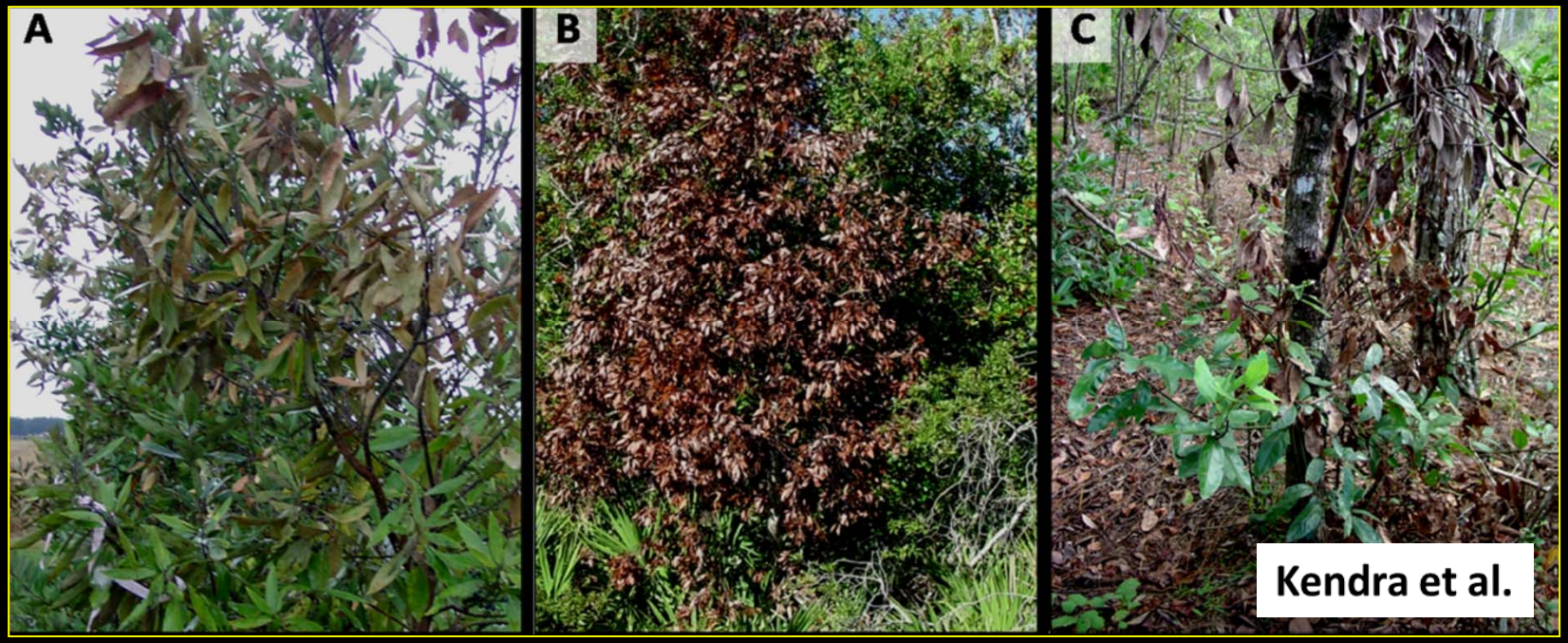
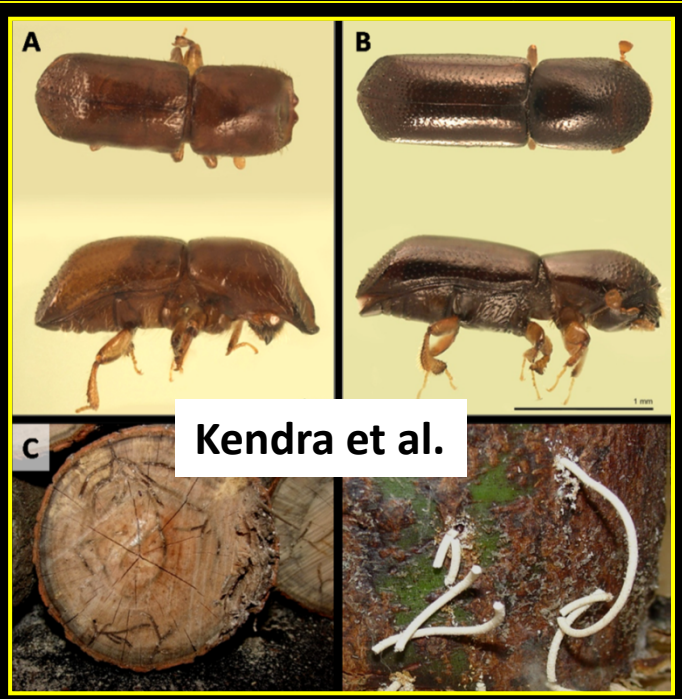
Diagnosis: Symptoms,


A
B
C
Kendra et al.


A
B
C
Kendra et al.


Mayfield

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 (+ cyclohexamide insenstitivity),

Pathogen

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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

Pathogen

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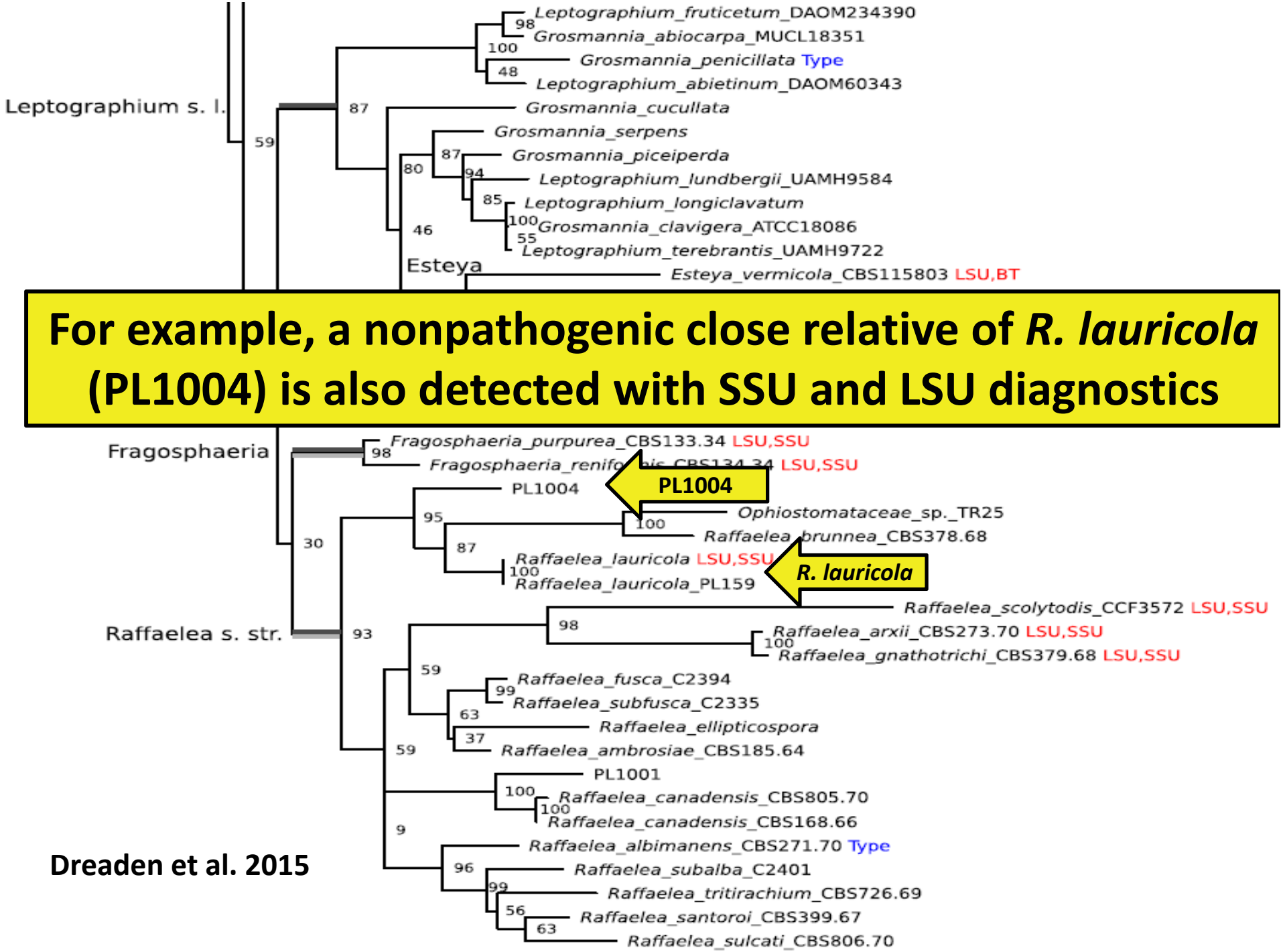
Raffaelea **spp. are confused taxonomically. They are nutritional symbionts of ambrosia beetles, and are predominantly not plant pathogens.**

Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU 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

Dreaden et al. 2015

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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

Dreaden, T. J., Davis, J. M., Harmon, C. L., Ploetz, R. C., Palmateer, A. J., Soltis, P. S., and Smith, J. A. 2014. Development of multilocus PCR assays for *Raffaelea lauricola*, causal agent of laurel wilt disease. Plant Dis. 98:379-383.

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

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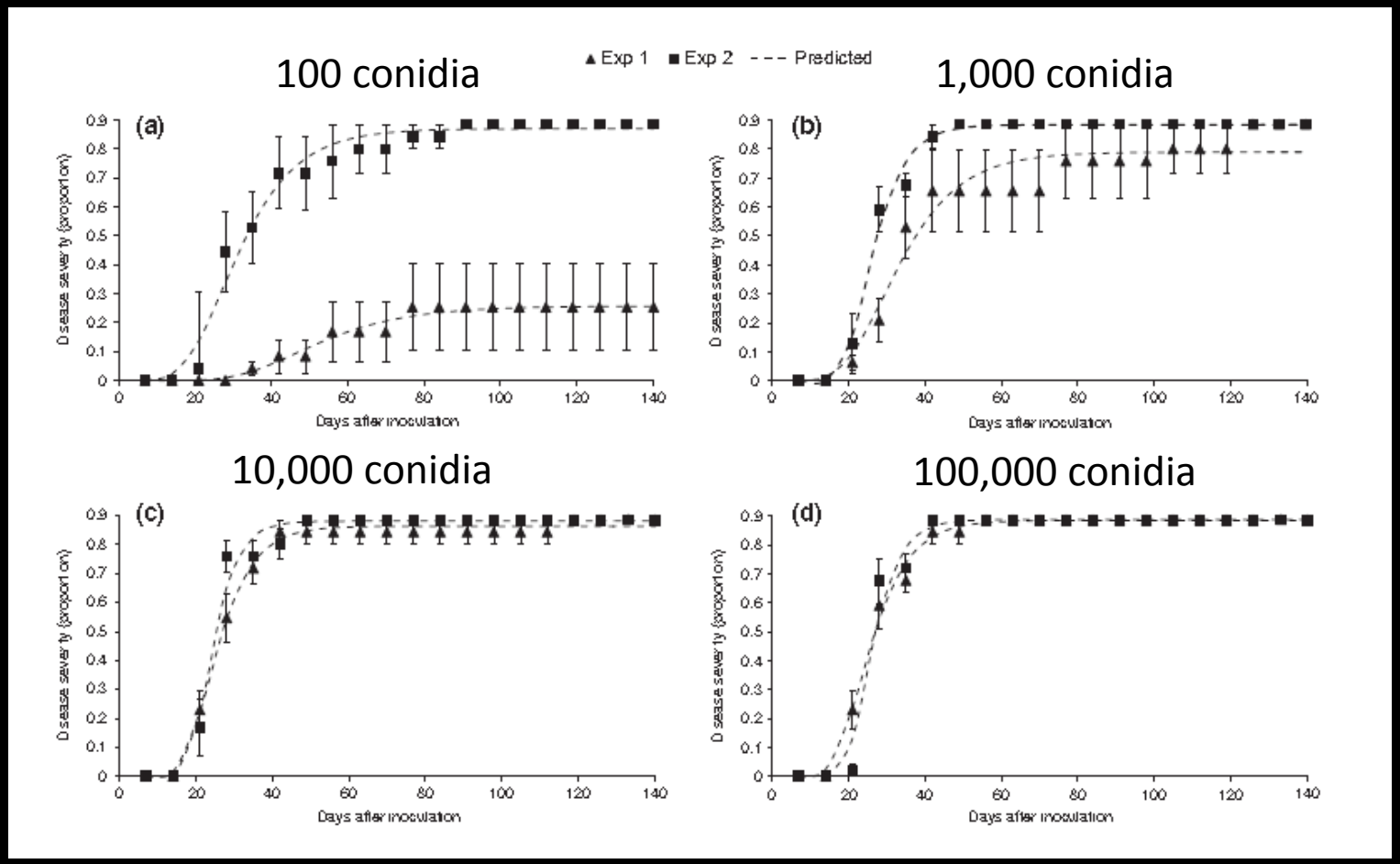
Diagnosis: Symptoms, cultural morphology, and molecular identity (SSU, LSU, SSRs) of the pathogen

Infection, colonization and host response

These are extremely sensitive pathosystems



Swampbay, *Persea palustris*

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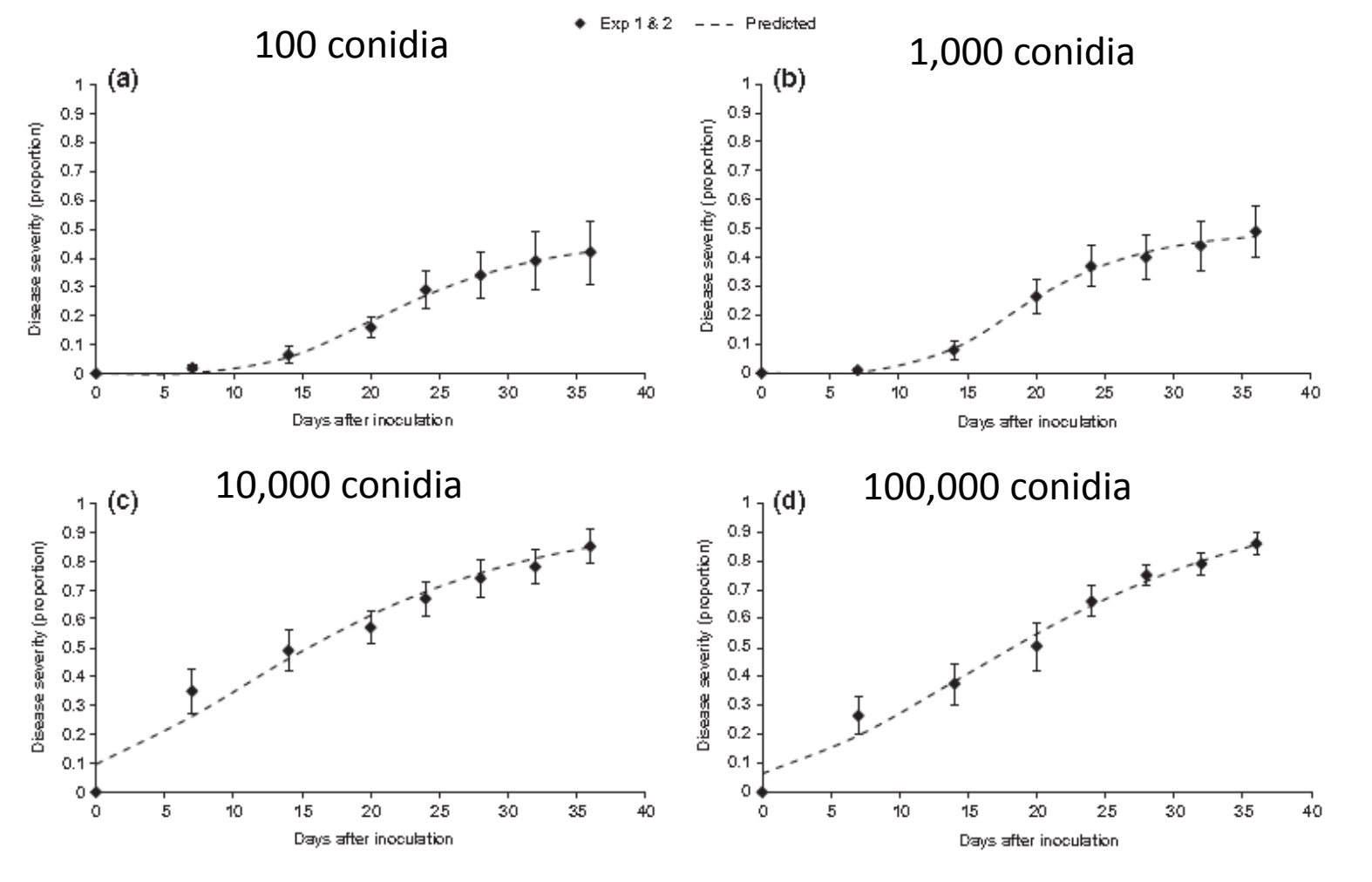
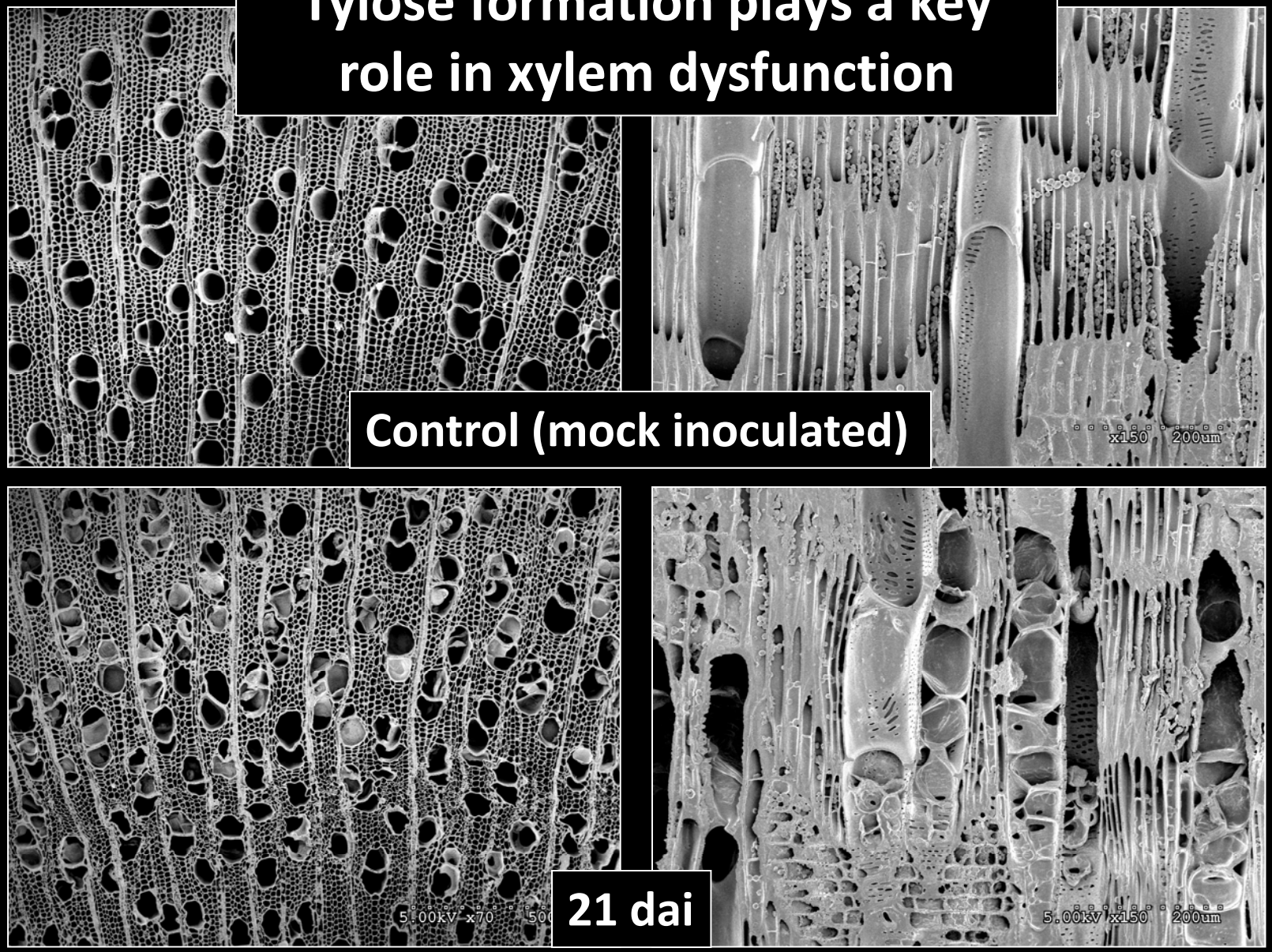
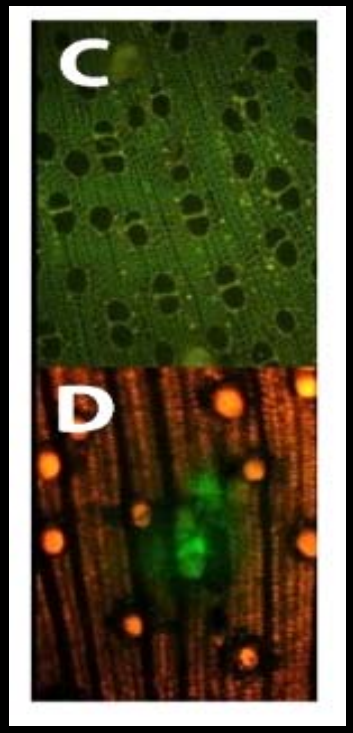


Avocado, *Persea americana*

Tylose formation plays a key role in xylem dysfunction

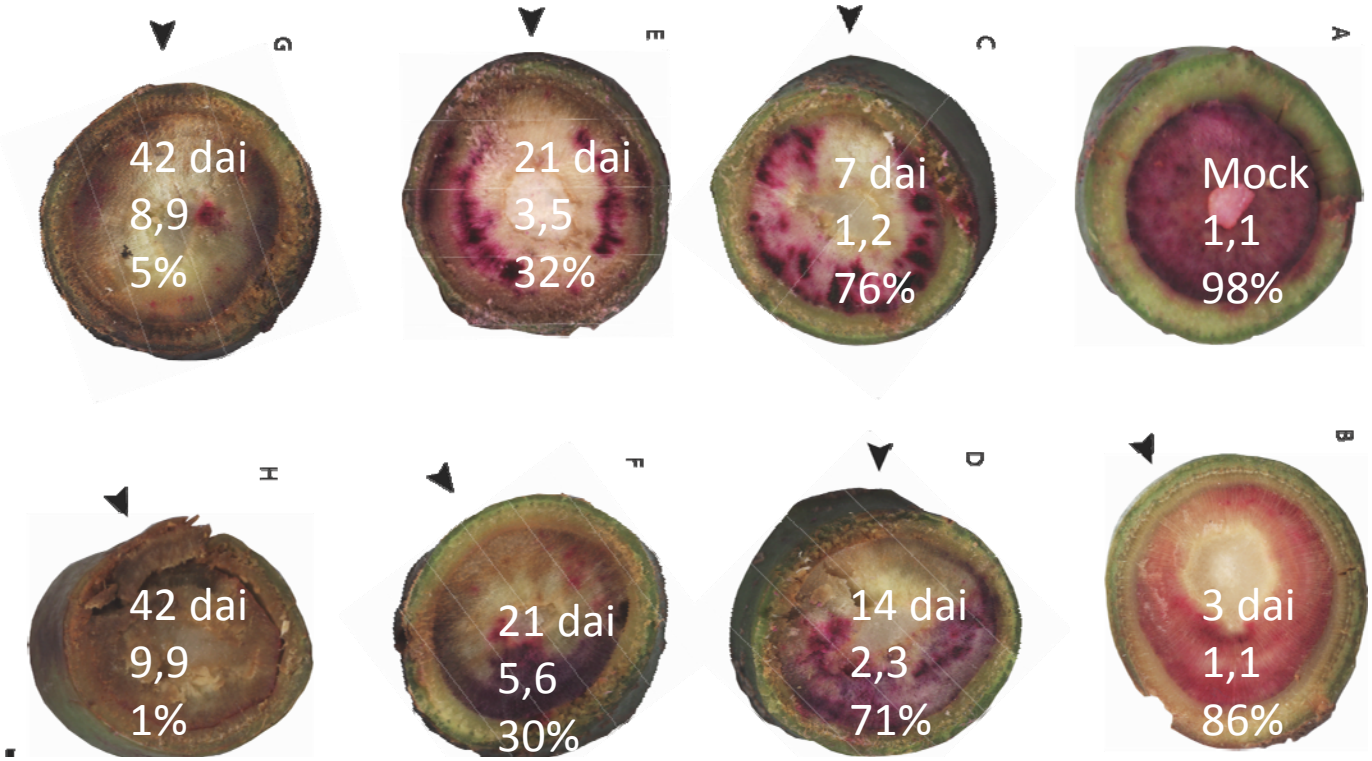




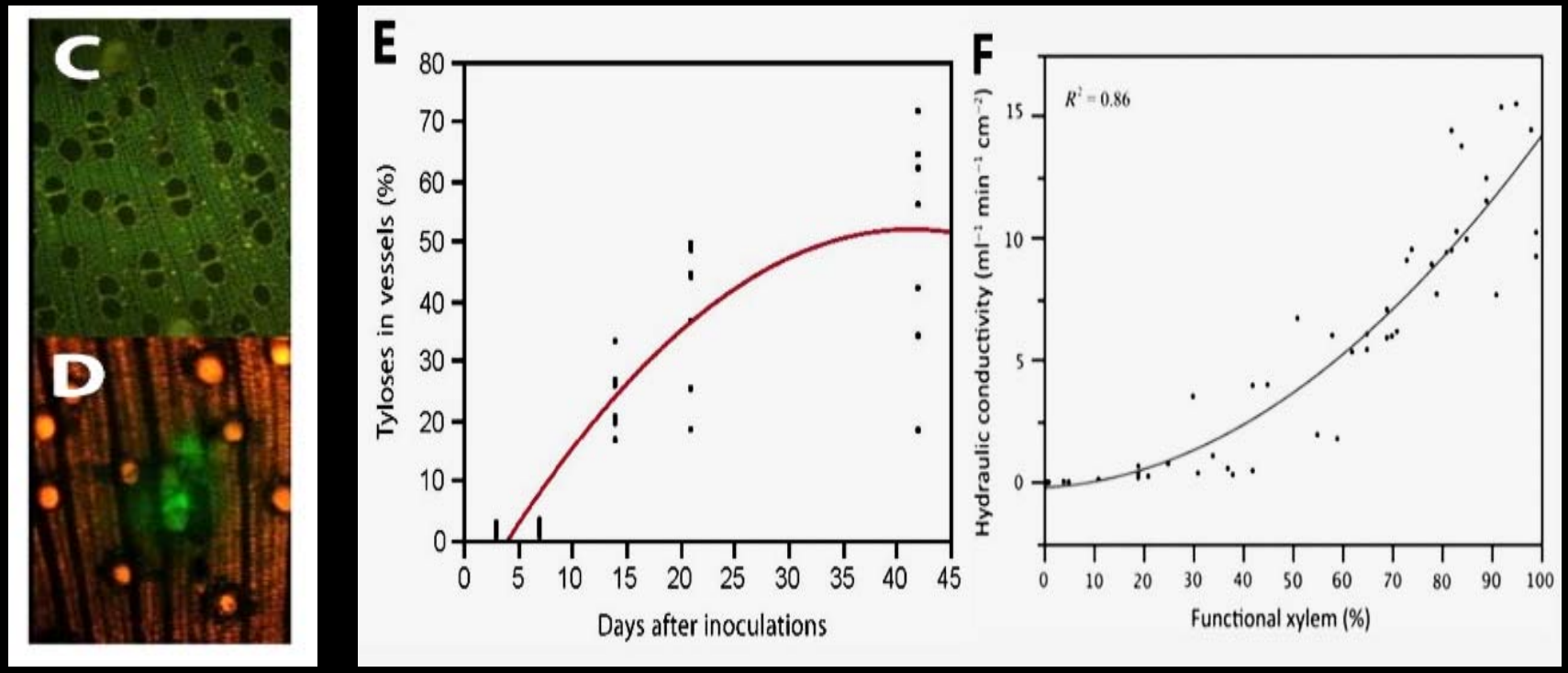
Raffaelea lauricola induces tylose formation in infected avocado trees, but is scarcely evident, microscopically. Ten days after inoculation, a gfp-marked strain of the pathogen is: (C) not visible, until (D) 3 days after a bioenrichment step.

Impact of laurel wilt on xylem function

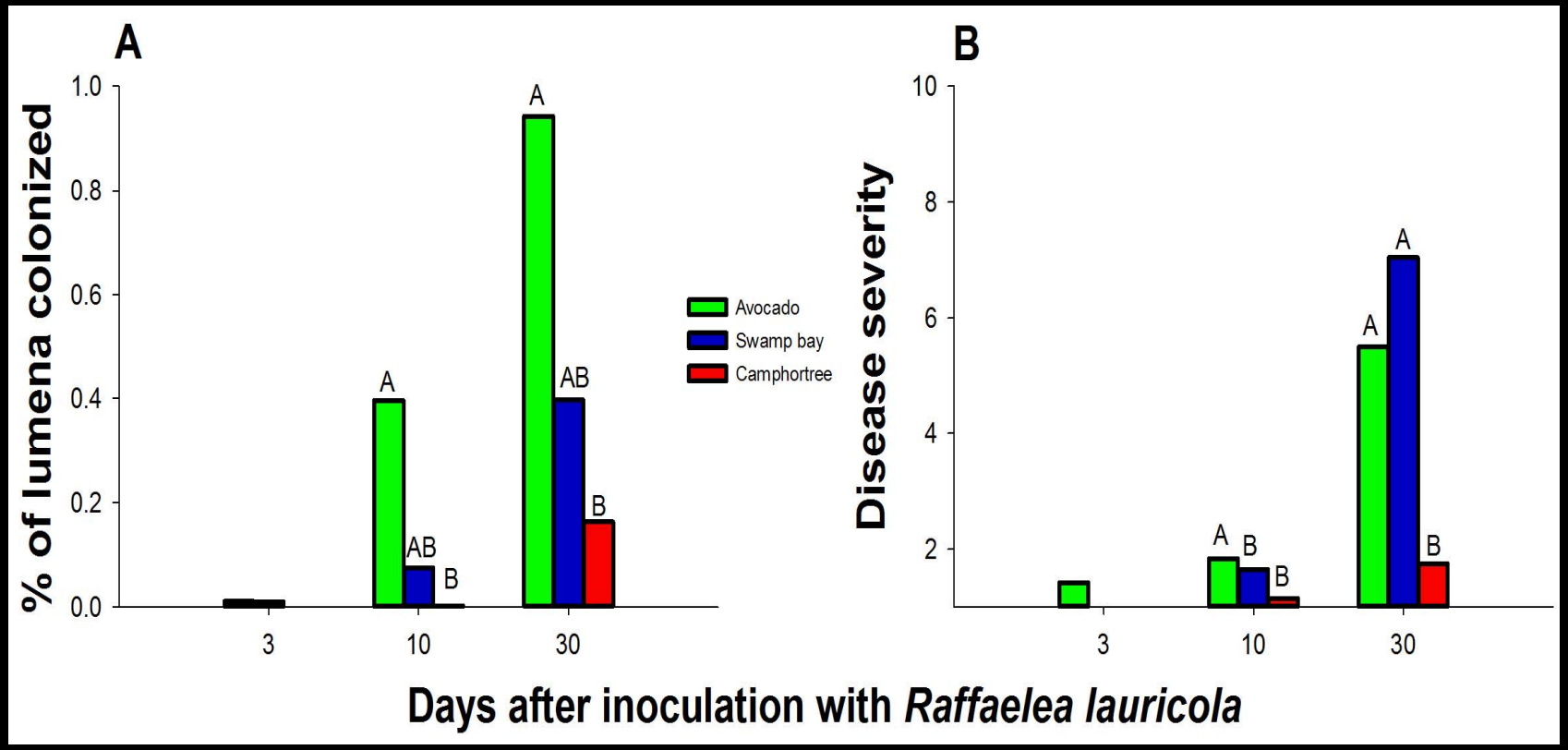
Functional xylem, acid fuchsin assay



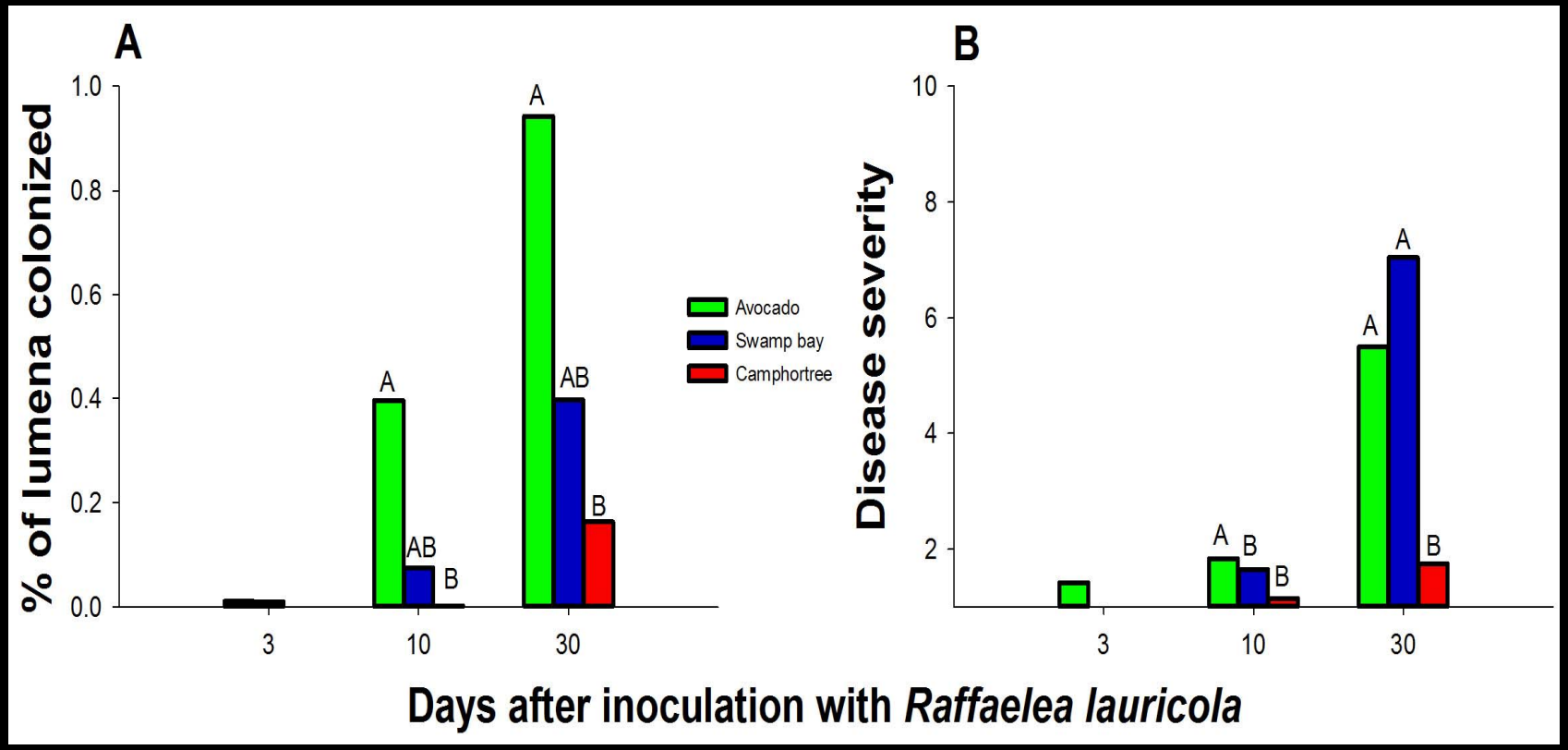
dai = days after inoculation. External, internal disease severity: 1 = no symptoms, 10 = dead or 100% symptomatic. % = functional relative xylem, based on acid fuchsin stain/uptake. Scale = 0.5 cm .
Point ▼ indicates inoculation location.



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 lumena 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?

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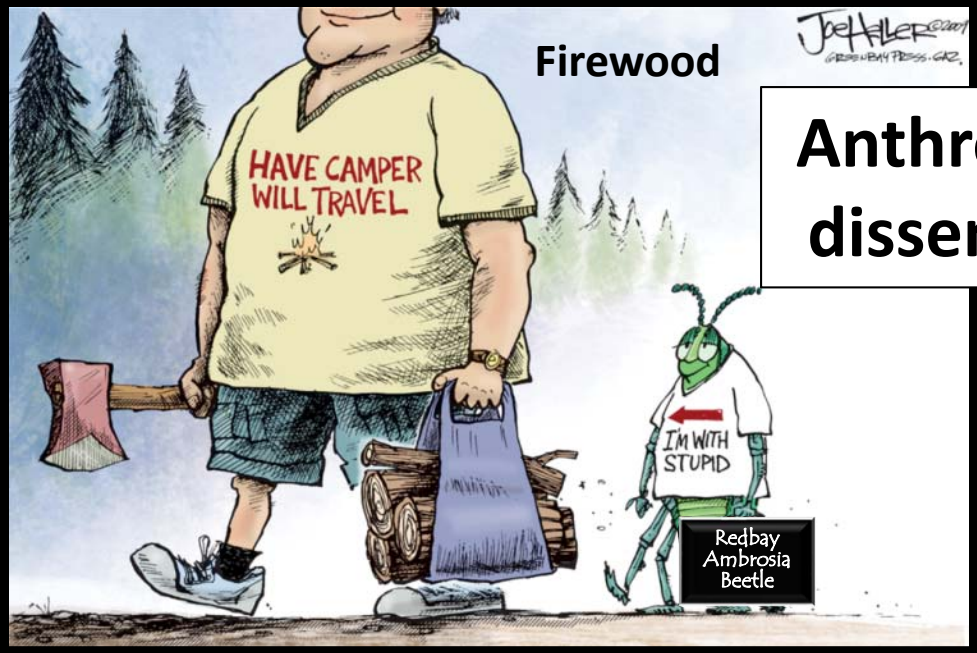
Infection, colonization and host response

Epidemiology

Epidemiology

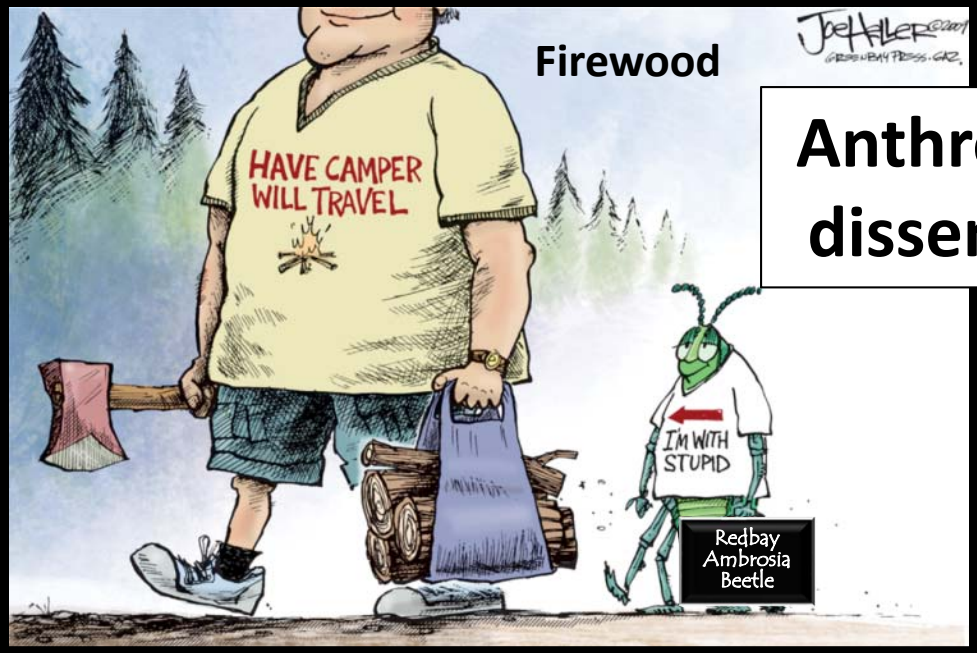
Anthropogenic dissemination

Anthropogenic dissemination

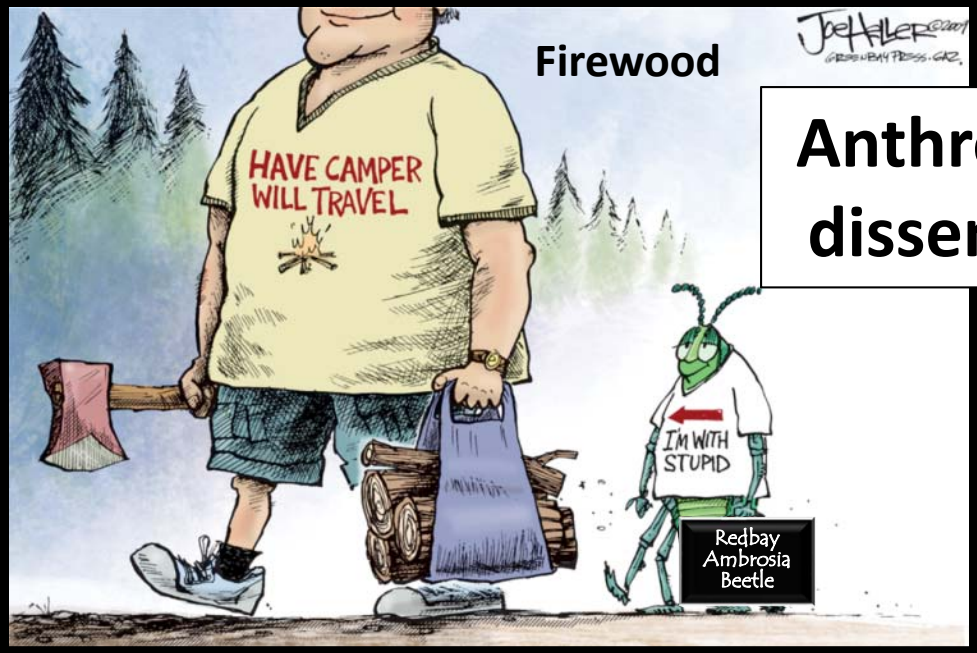


Anthropogenic dissemination

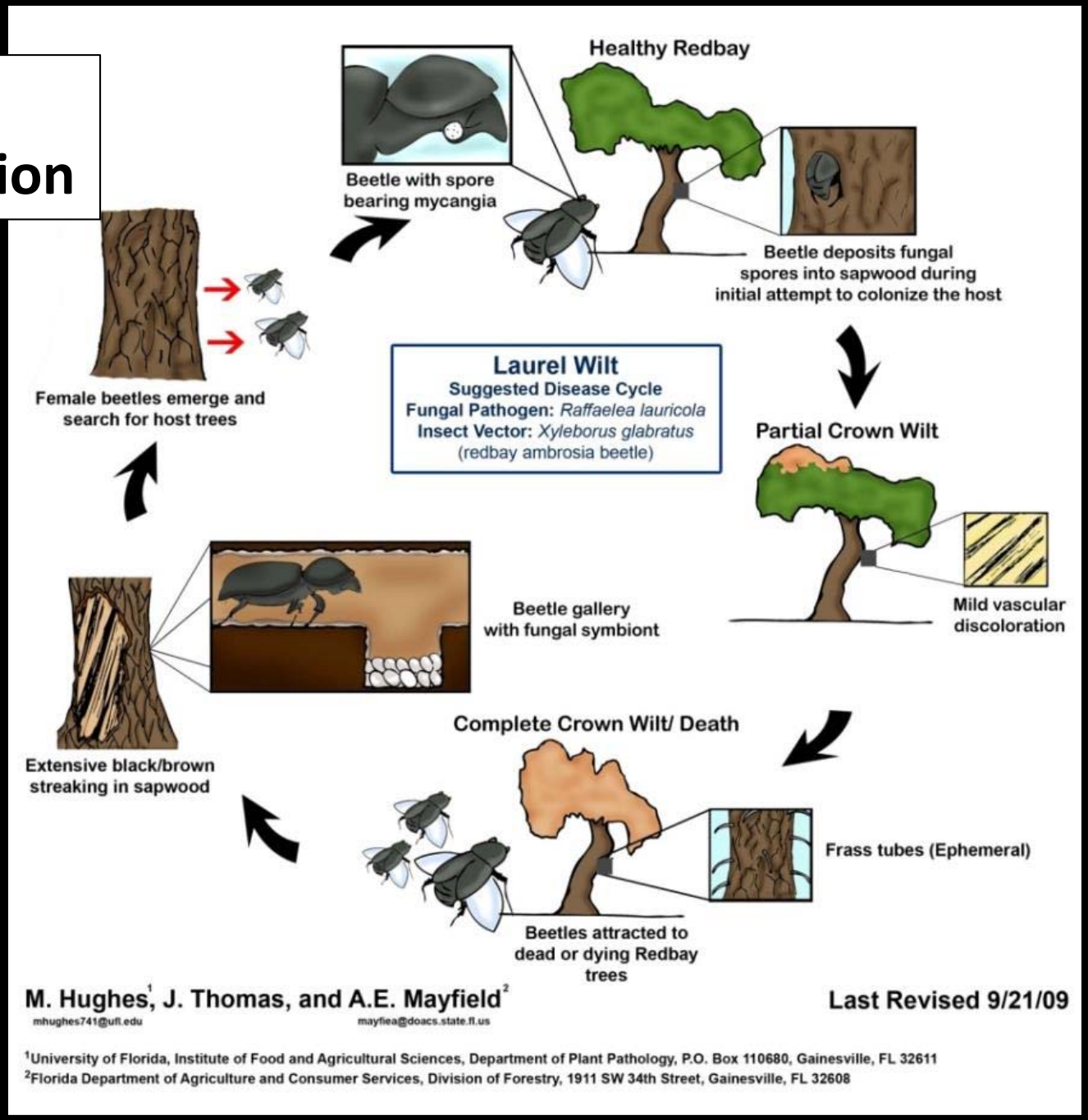
Anthropogenic dissemination

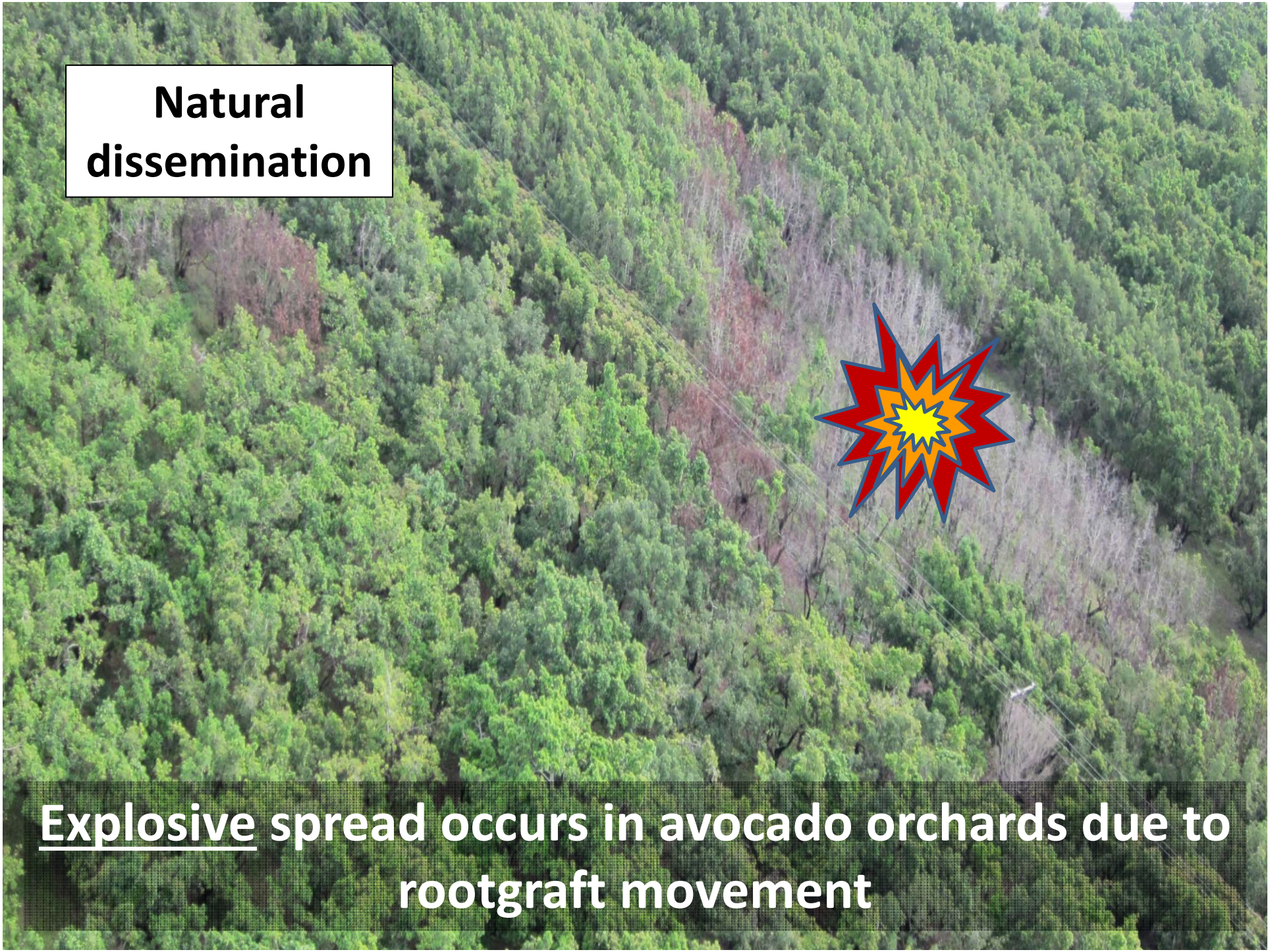


Anthropogenic dissemination

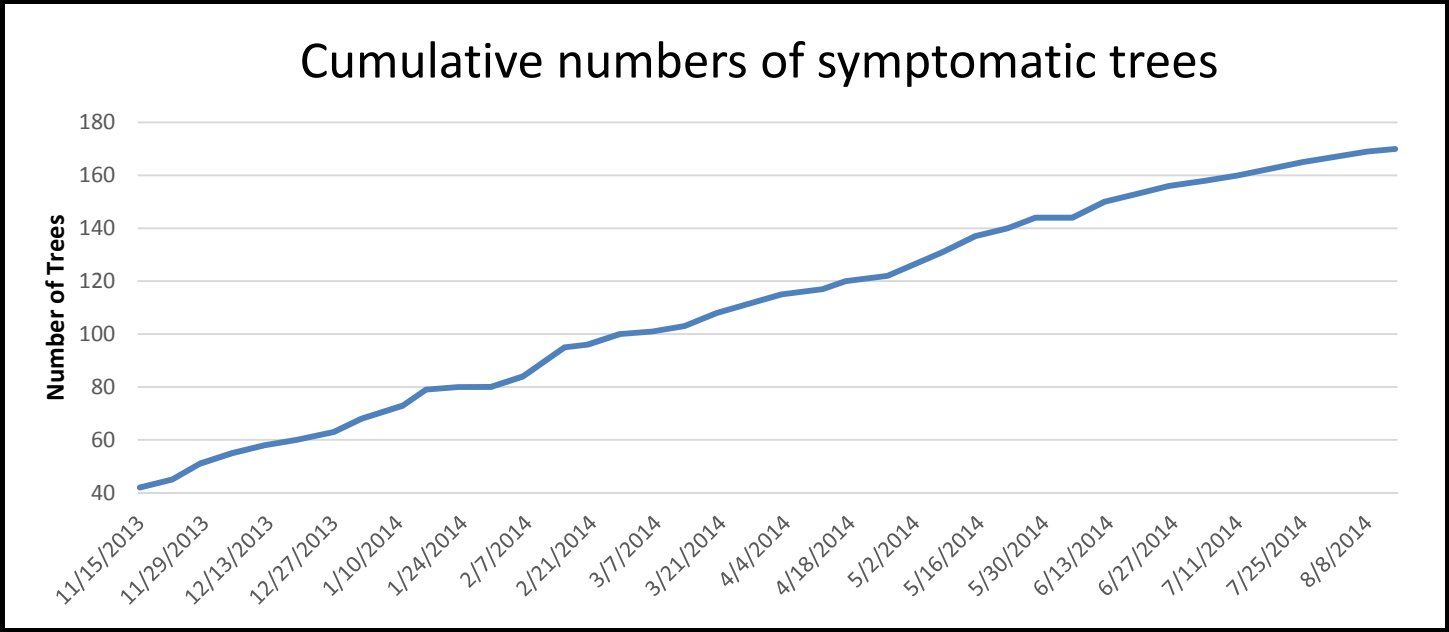


Natural dissemination




Natural dissemination
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})

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

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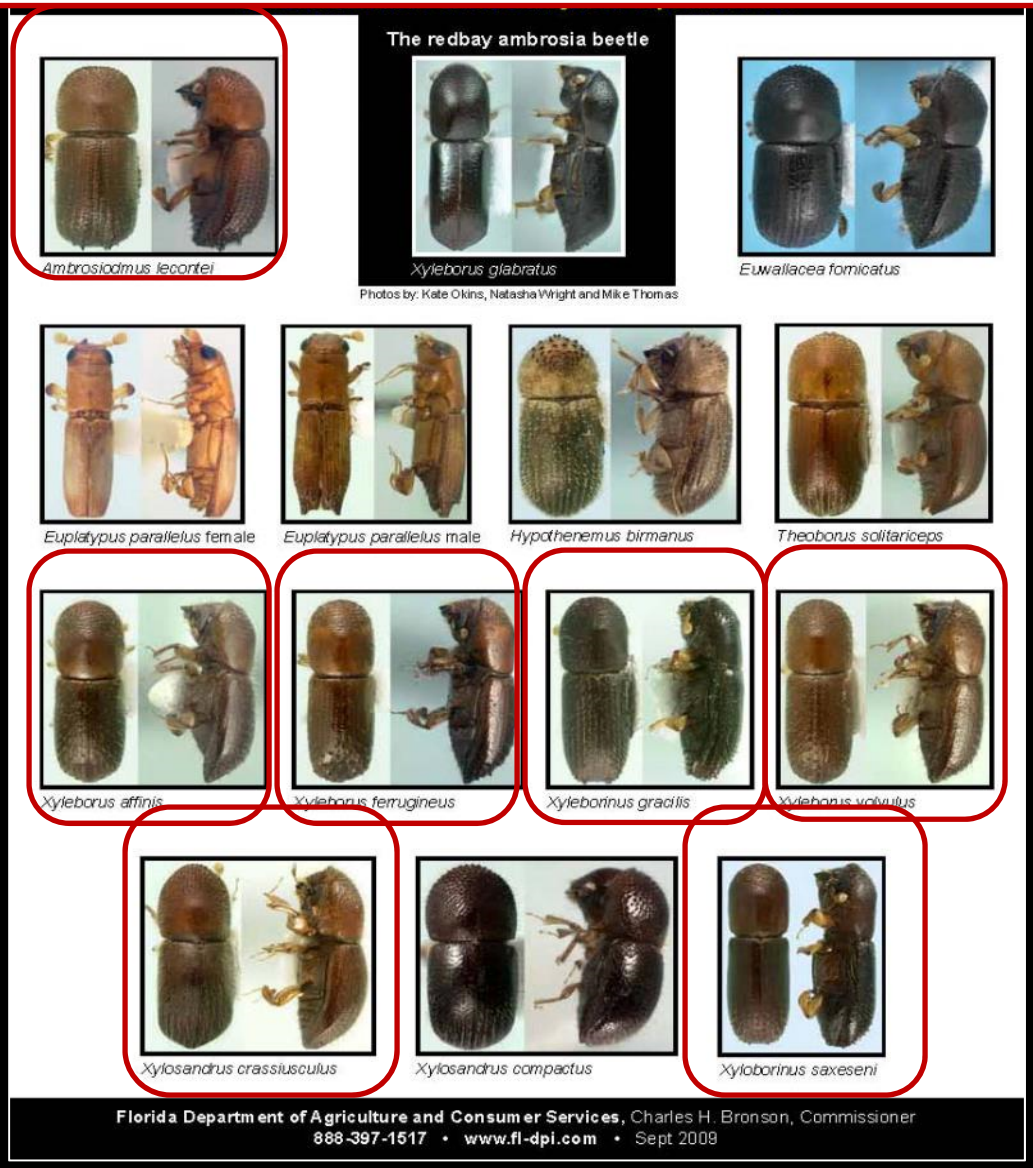
Epidemiology

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

Species	Reared from laurel wilt-affected bolts of swamp bay, *Persea palustris*					Reared from laurel wilt-affected bolts of avocado, *Persea americana*				
	n	No. w *R. lauricola*	% beetles w *R. lauricola*	CFU mean	CFU range	n	No. w *R. lauricola*	% beetles w *R. lauricola*	CFU mean	CFU range[z]
Xyleborus glabratus	50	43	86	2783.3	0–7800	0 *	n/a	n/a	n/a	n/a
Xyleborus affinis	41	5	12	1	0–20	16	0	0	0	0
Xyleborus bispinatus	n/a	n/a	n/a	n/a	n/a	5	5	100	40.8	4 - 80
Xyleborus ferrugineus	118	70	59	33	0–118	2	2	100	5	4 - 6
Xyleborus volvulus	39	20	51	28.4	0–100	53	10	19	30.0	0 - 1140
Xyleborinus gracilis	52	26	50	100.6	0–1240	10	0	0	0	0
Xyleborinus saxeseni	68	2	3c	1.5	0–60	51	0	0	0	0
Xylo. crassiusculus	39	1	3	2.6	0–100	24	1	4	1	0 - 360
Ambro. devexulus	25	0	0	0	0	0	n/a	n/a	n/a	n/a
Ambro. lecontei	41	0	0	0	0	0	n/a	n/a	n/a	n/a
Hypothenemus sp.	0	n/a	n/a	n/a	n/a	1	0	0	0	0
Totals/means	473		35.3			162		11.1		

*** 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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- 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

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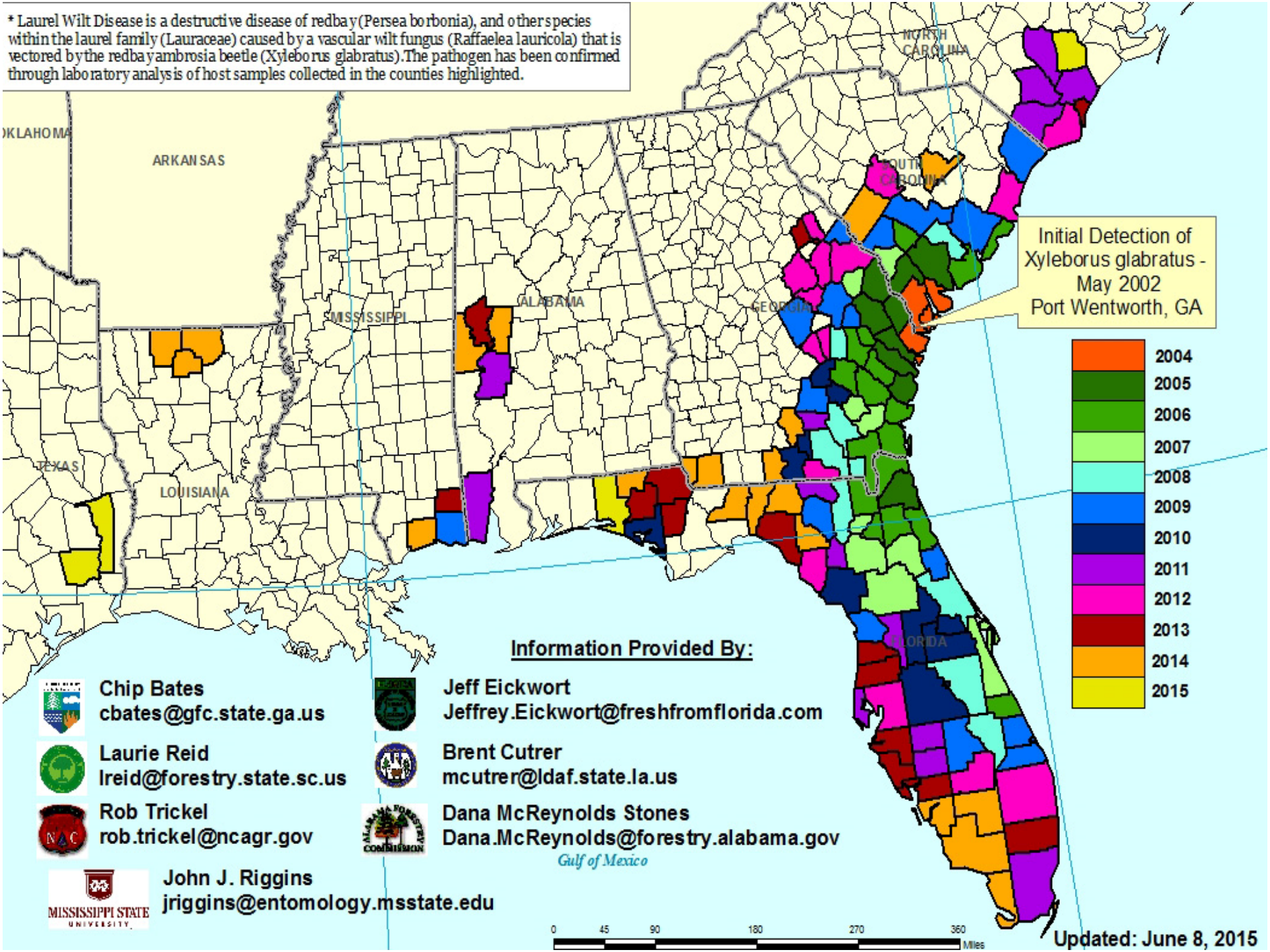
  - Natural spread

    ambrosia beetles – *Xyleborus glabratus* 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 redbay (Persea borbonia), and other species within the laurel family (Lauraceae) caused by a vascular wilt fungus (Raffaelea lauricola) that is vectored by the redbay ambrosia beetle (Xyleborus glabratus). The pathogen has been confirmed through laboratory analysis of host samples collected in the counties highlighted.
OKLAHOMA
ARKANSAS
TEXAS
LOUISIANA
MISSISSIPPI
ALABAMA
GEORGIA
SOUTH CAROLINA
NORTH CAROLINA
FLORIDA
Initial Detection of Xyleborus glabratus - May 2002 Port Wentworth, GA
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Information Provided By:
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Updated: June 8, 2015

Special thanks to:

Tyler Dreaden. Pathogen phylogeny and detection

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Alina Campbell. Host x pathogen interactions

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