Laurel Wilt Epidemiology and Management

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July 29-August 2, 2019: Laurel wilt-ambrosia beetle seminar series, California





Data presented here is the product of ~10 years research conducted by many researchers at UF, UF-TREC, and collaborators from different institutions



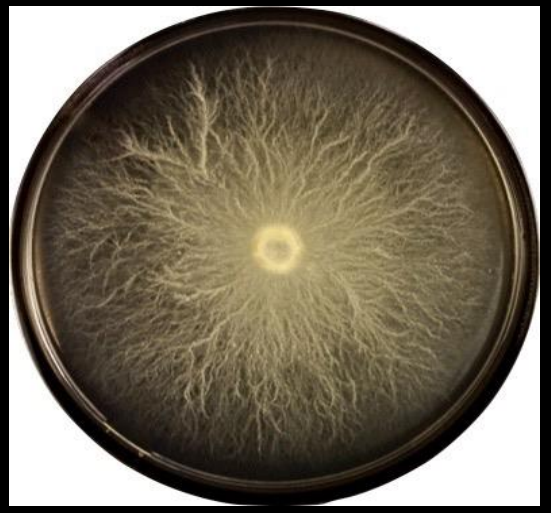
University of Florida Plant Pathology

UF IFAS UNIVERSITY of FLORIDA

SFRC School of FOREST RESOURCES & CONSERVATION

Laurel Wilt of Avocado (LW) – Disease Complex

Raffaelea lauricola
(causal agent)

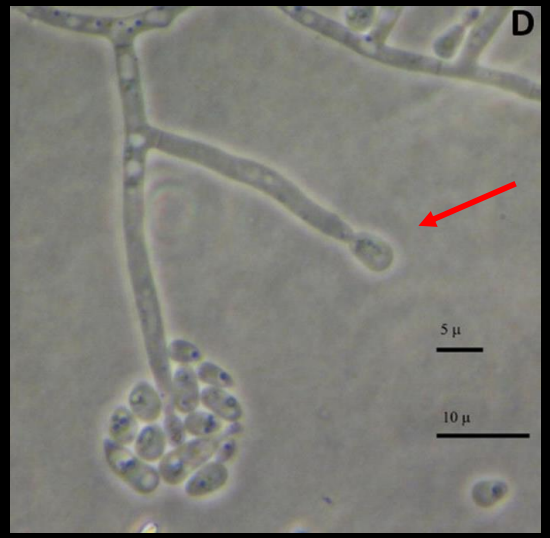


Xyleborus glabratus
(Redbay ambrosia beetle, original vector)

Lauraceae Family

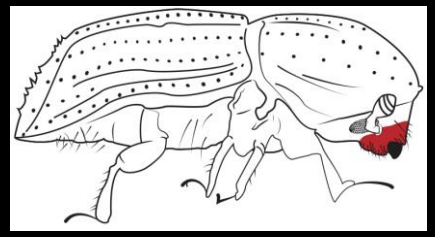
Laurel Wilt of Avocado (LW) – Disease Complex

Raffaelea lauricola
(RL, causal agent)

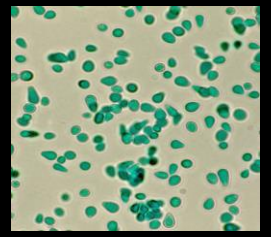


- Vascular pathogen (invades host's xylem)
- Primary nutritional symbiont of the Redbay Ambrosia Beetle, *Xyleborus glabratus*
- Carried within the beetle's mycangia ("pockets") and inoculated into the trees by the beetle

Xyleborus glabratus

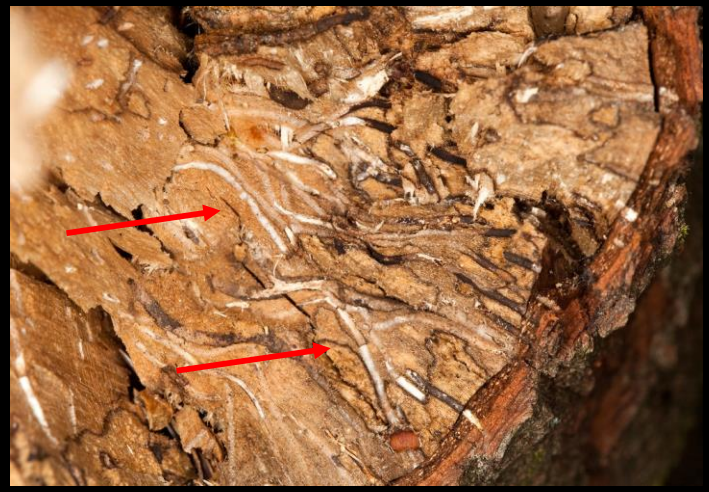


Pre-oral mycangia



RL yeast

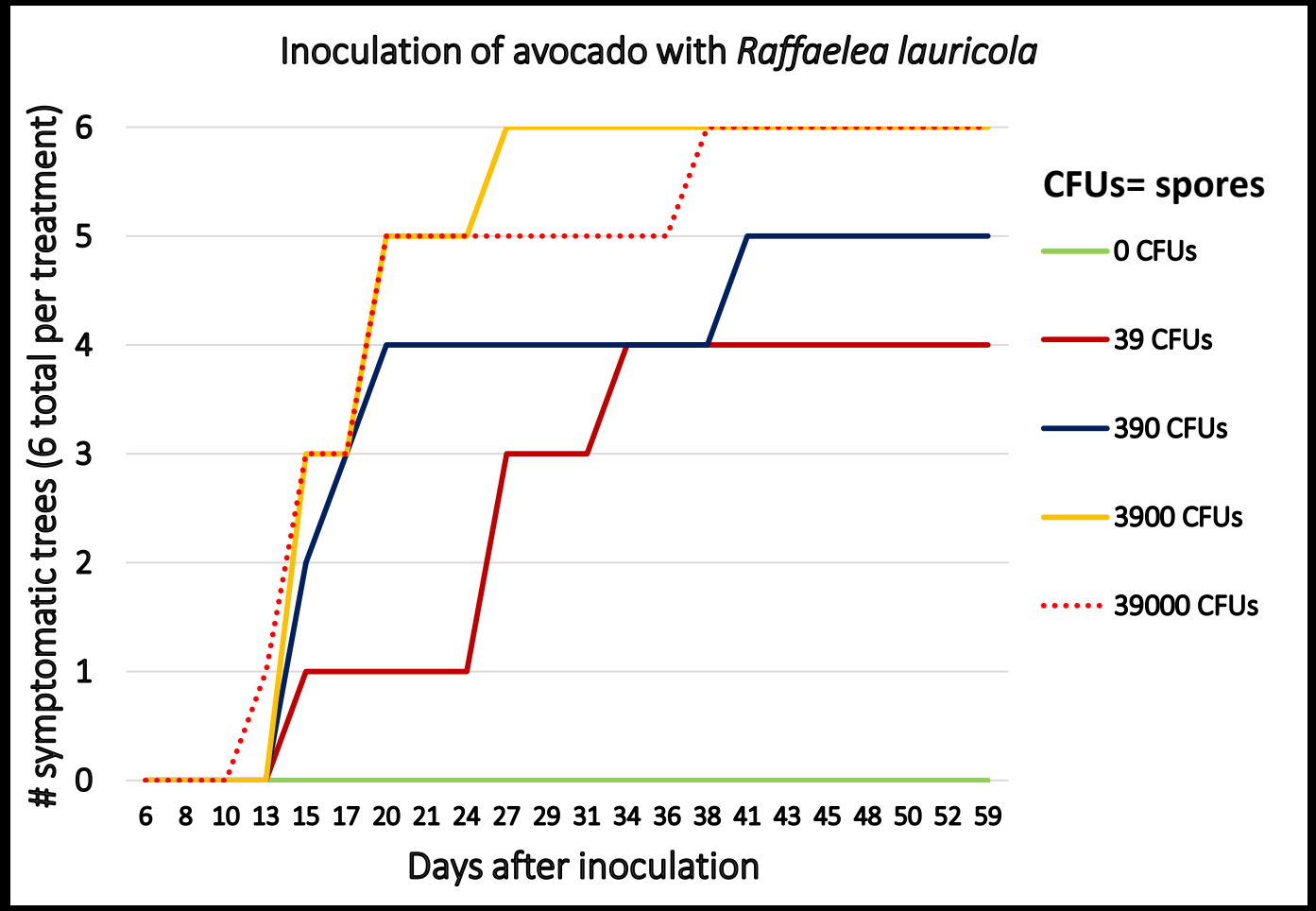
"Blue staining Fungi"



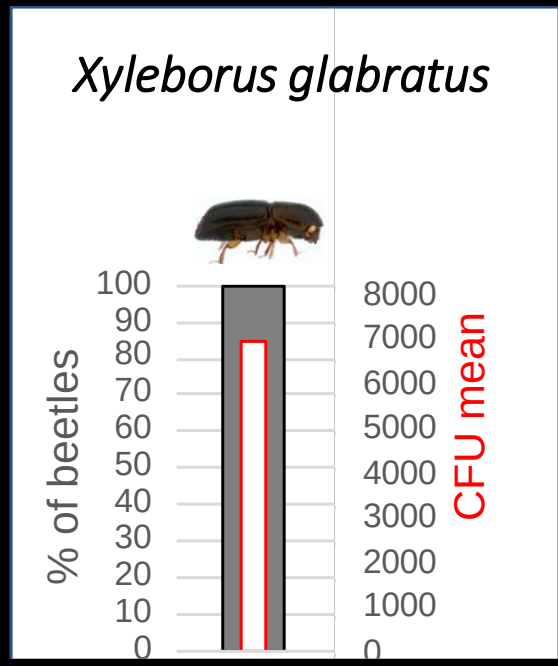
Swampbay tree trunk cross-section showing the Redbay ambrosia beetle's galleries filled with the fungus RL

Gallery picture: USDA, beetle drawing R. Fernandez

RL is a Very Virulent Fungus!



One beetle can inoculate enough RL to kill a tree



Average beetle carries > 6,000 spores

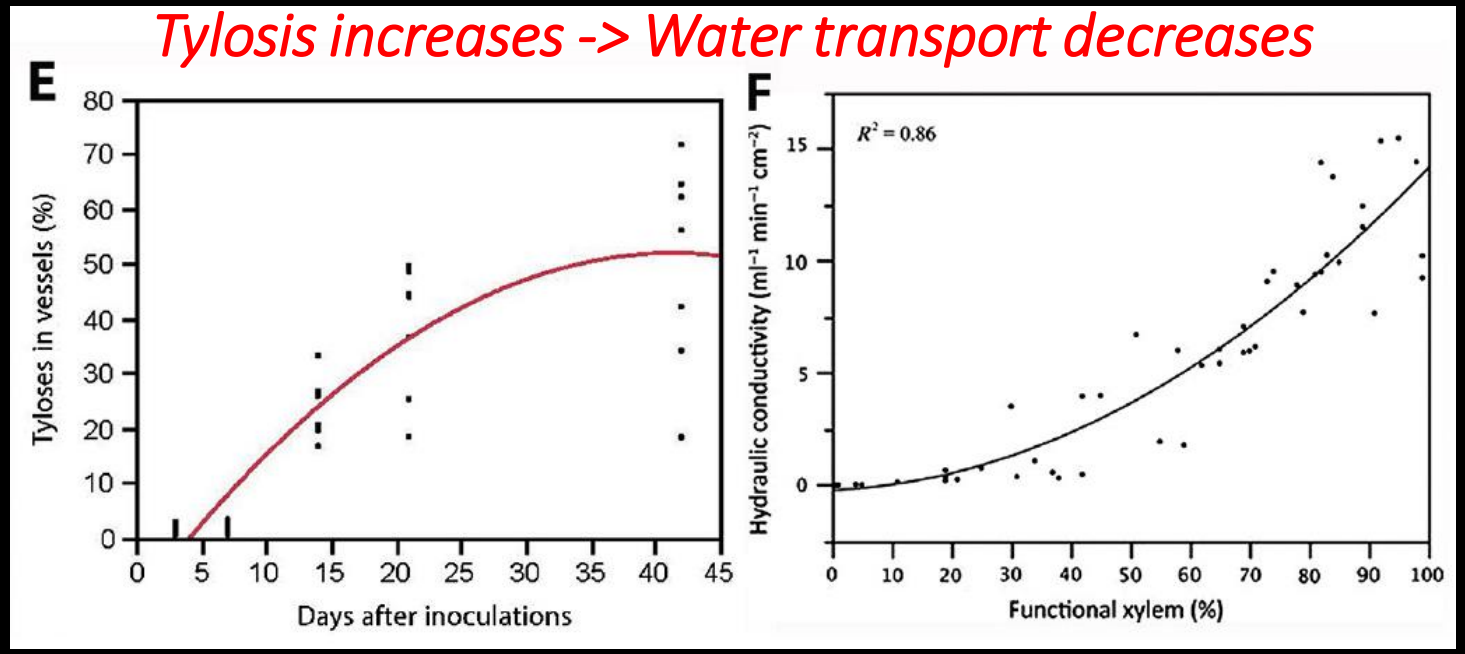
- 390 spores induced symptoms in 5 of 6 of trees inoculated
- 39 spores induced symptoms in 4 of 6 of trees inoculated
- Low CFUs required for disease development increases risk from alternative vectors carrying lower titers of the pathogen (**Dr. Carrillo's talk)

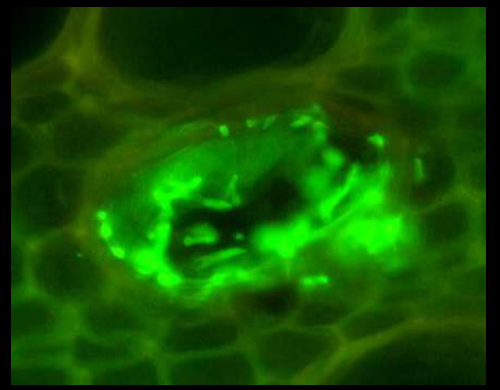
Host x Pathogen Interaction = Vascular Pathogen that induces the formation of Tylosis

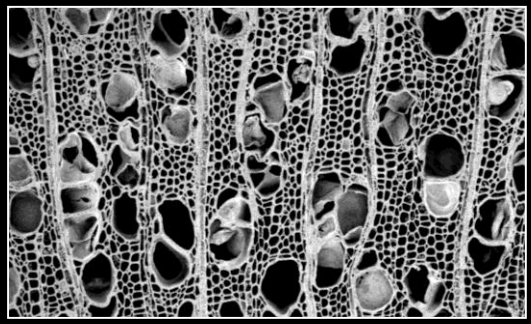
Dye-binding assay: burgundy to pink coloration indicates functional xylem
IS = Internal Symptoms

DISEASE PROGRESS

Tylosis increases -> Water transport decreases







Extensive Damage to Natural Habitats

REDBAY

SASSAFRAS

PONDBERRY

PONDSPICE

SILKBAY

SWAMP BAY

- An estimated **320 million trees** – nearly one-third of all Redbays – have been killed
- The disease has reached the Everglades National Park
- There are more than 30 species of Native Lauraceae in USA, many of which are susceptible to LW

Sources:
Hughes et al. 2017. DOI 10.1007/s10530-017-1427-z
https://www.srs.fs.usda.gov/futures/summary-report/web/summaryreport-13.htm
https://www.dontmovefirewood.org/pest_pathogen/laurel-wilt-html/

Extensive Damage to Avocado Groves

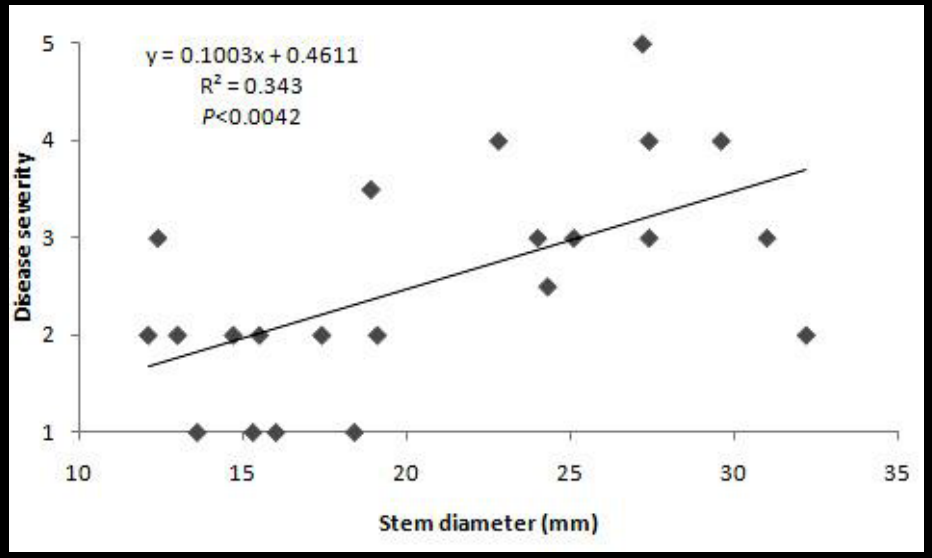
The FL avocado industry has **lost over 120,000 trees** due to laurel wilt since the introduction of the disease

TREC research on LW: https://trec.ifas.ufl.edu/RAB-LW-2/RAB_Research.shtml

All avocado cultivars are susceptible but vary on how rapid they decline

- Disease rating 1-10
- Experiments conducted in 3 gall. potted plants

Disease progress is faster in larger trees



- ALL tested cultivars are susceptible
- Response to the infection varies among cultivars with West Indian cultivars declining more rapidly
- Dr. Schaffer's talk*

Response of different avocado cultivars and genomes to laurel wilt		
Cultivar	Race	Genome mean
'Ettinger'	GxM	Guatemalan x Mexican 2.8 b
'Hass'	GxM	
'Pinkerton'	GxM	
'Winter Mexican'	GxM	
'Bacon'	G	Guatemalan 2.5 b
'Marcus Pumpkin'	G	
'Reed'	G	
'Brogdon'	GxMxWI	-
'Oro Negro'	MxWI	-
'Beta'	GxWI	Guatemalan x West Indian 3.9 ab
'Choquette'	GxWI	
'Hall'	GxWI	
'Lula'	GxWI	
'Miguel'	GxWI	
'Monroe'	GxWI	
'Tonnage'	GxWI	
'Bernecker'	WI	West Indian 4.8 a
'Catalina'	WI	
'Day'	WI	
'Donnie'	WI	
'Hardee'	WI	
'Pollack'	WI	
'Russell'	WI	
'Simmonds'	WI	

2008: 6 reps; 2009: 10 reps; 2010: 12 reps. Ploetz et al. 2012.

Disease Symptoms

External

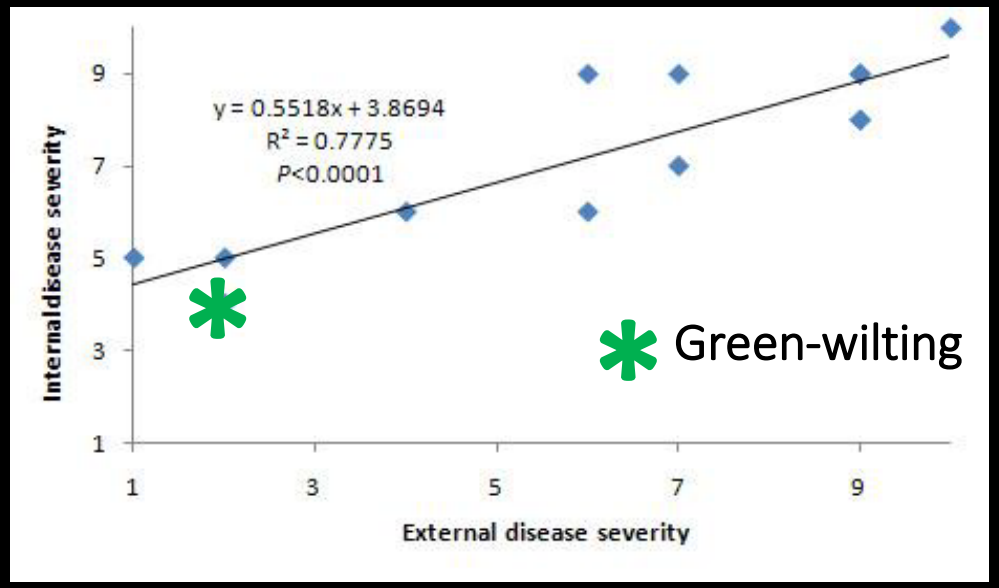
Internal

- The earliest external symptom green wilted leaves in sections of the canopy
- Green-leaf wilting is quickly followed by desiccation and browning of leaves, which remain attached to the plant stems
- Stem and limb dieback follow and eventually the entire tree declines and dies
- Frequently, trunks and/or limbs may have numerous small diameter holes with sawdust tubes
- Typical of vascular wilts, woody xylem becomes discolored and nonfunctional, and foliage subsequently dies due to a lack of water
- Underneath the bark, the normally cream- white sapwood may have dark blueish-black streaks

9-day interval

Green wilting → branch dieback → tree decline

Disease progress is very fast (within weeks), but we do not have an exact timeline
(multiple factors: inoculum concentration, infection mode, avocado cultivar, temperature, tree health)



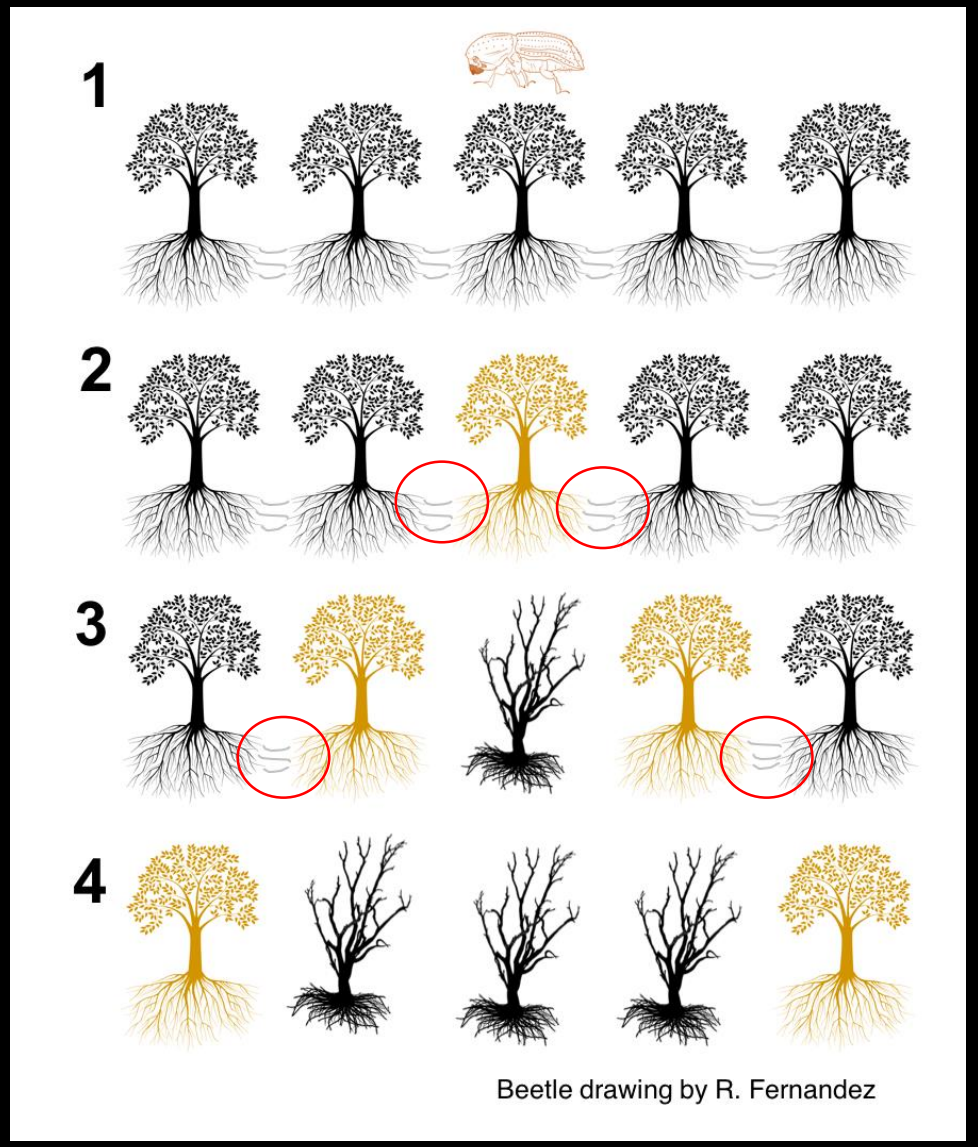
Internal symptoms (clogging of the xylem vessels) appear BEFORE external symptoms (wilting)

Disease Cycle in Avocado Groves: Beetle inoculation + root graft transmission

- First inoculation by an ambrosia beetle
- Pathogen spreads to the adjacent trees through root-grafts
- Multiple dead trees in a row
- Spread between rows are less likely but have been reported

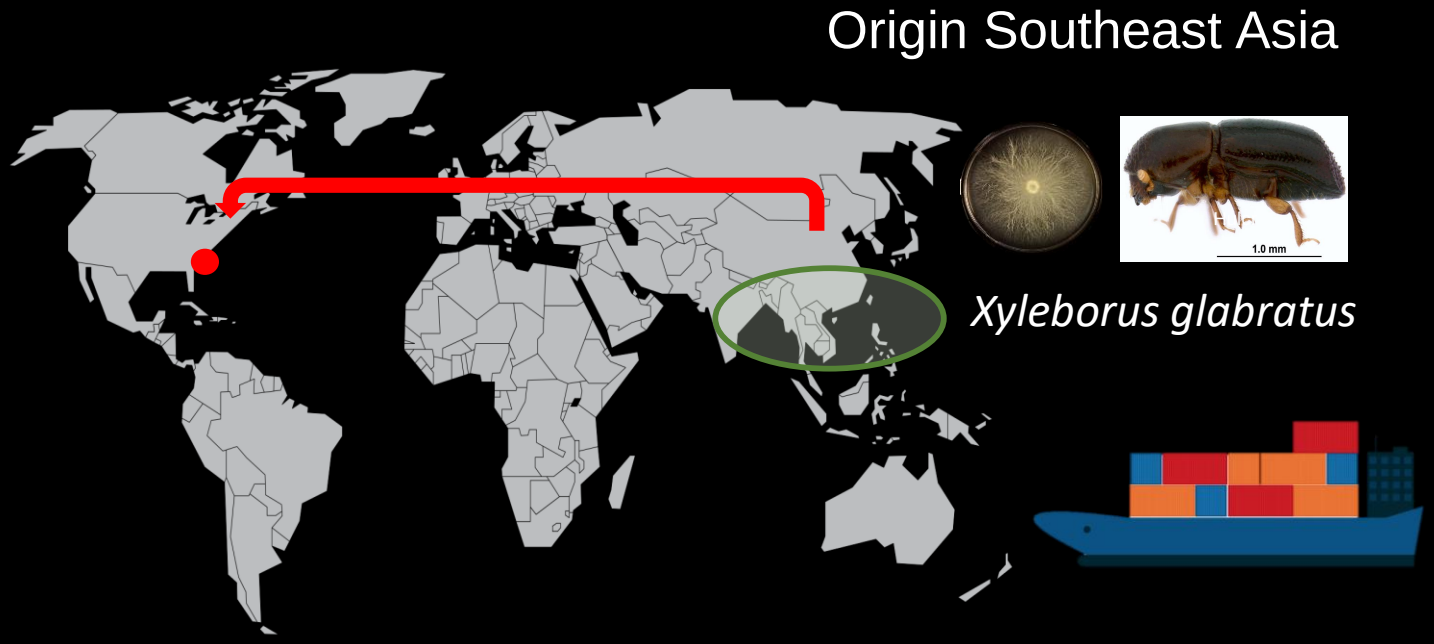
Important Unanswered Questions:

- Likelihood of root-grafted trees in a grove
- Timeline from beetle inoculation to root-to-root transmission to healthy adjacent trees. Does it varies based on grove age, soil type, management, cultivar?
- What are the characteristics of disease progress (time x severity) beetle inoculation vs. root-to-root transmission

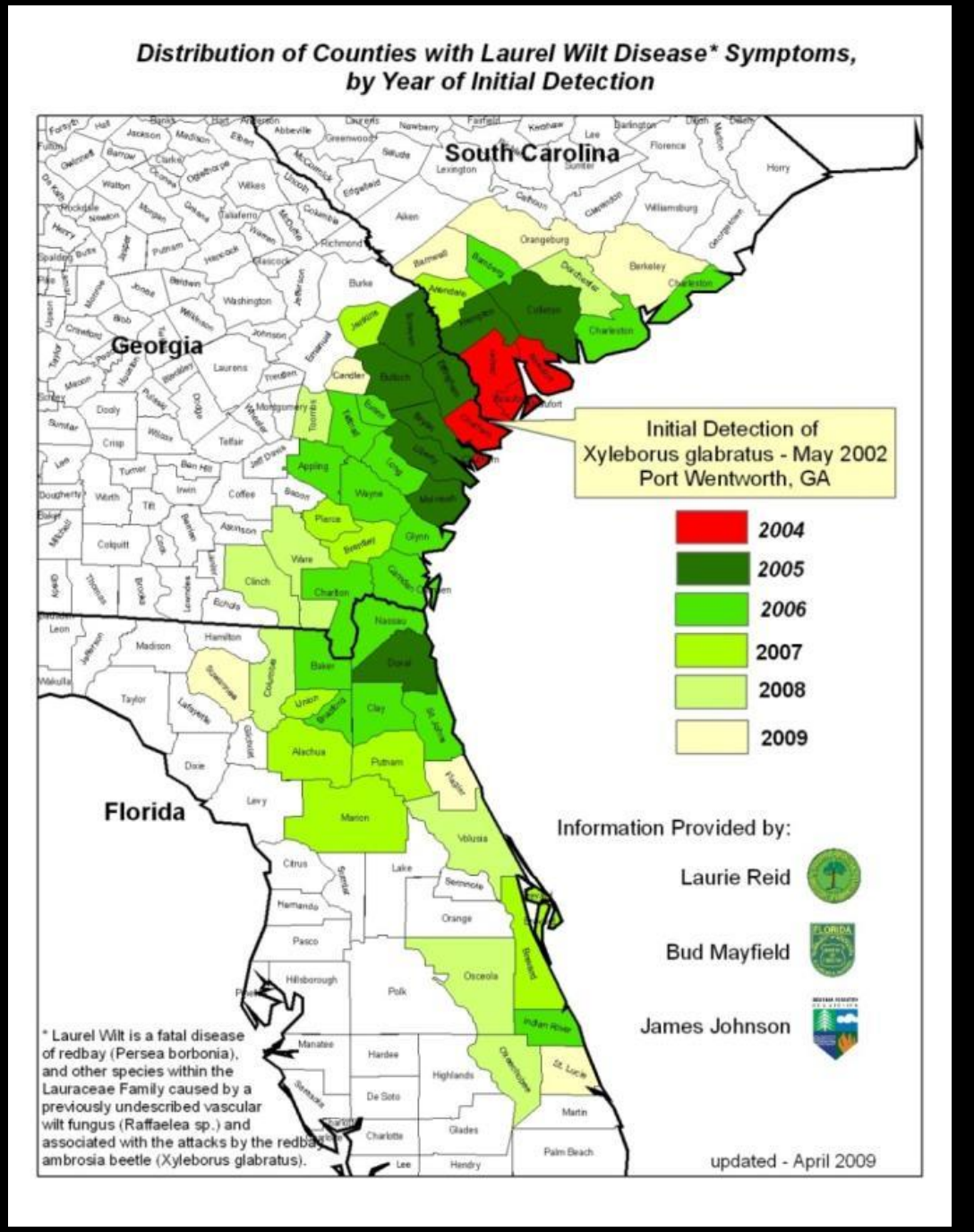


- Multiple outbreaks within a grove
- In 6 months –if no management is applied- more than 100 trees can be lost

Single Introduction Event into US

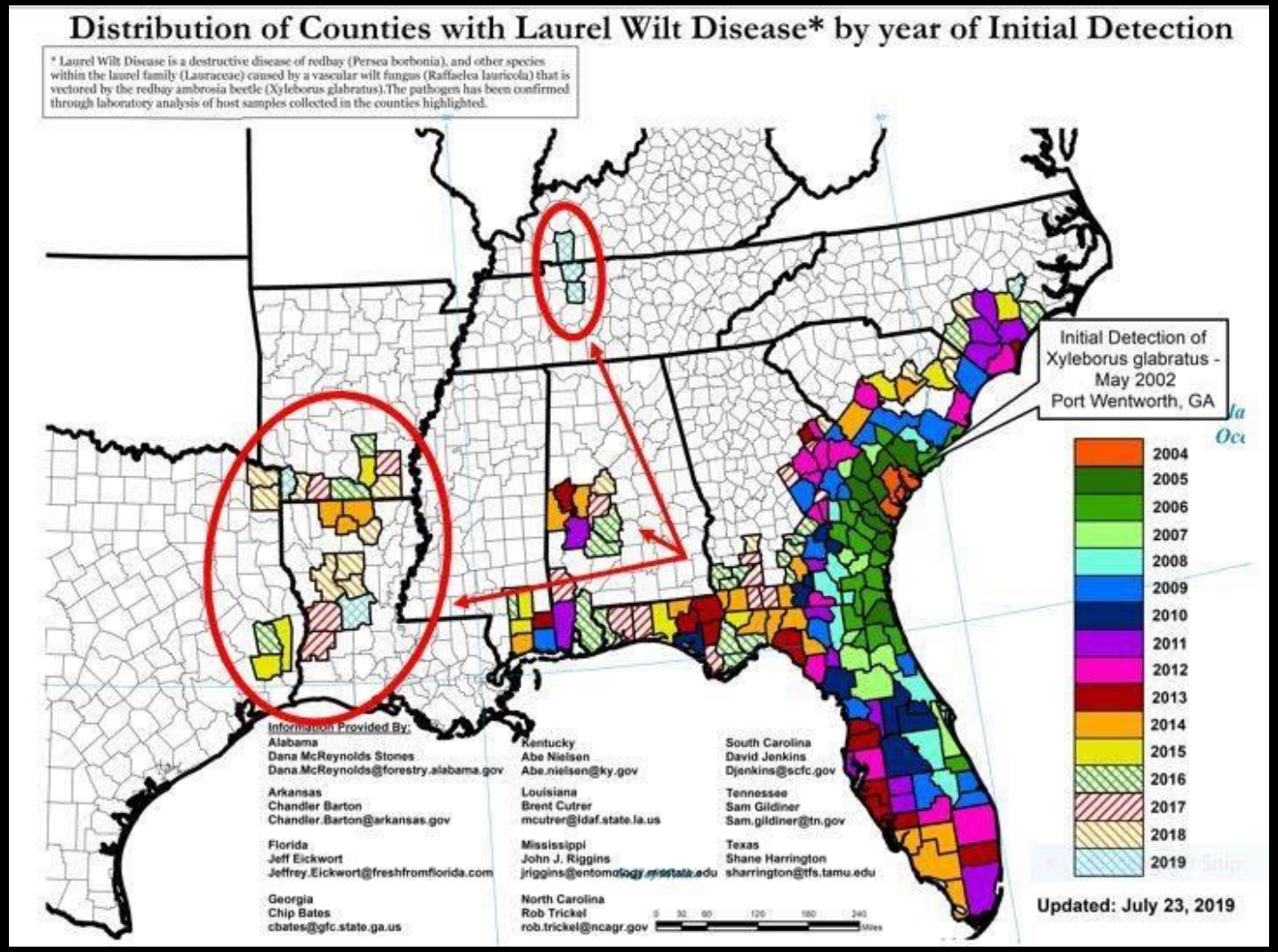


- Untreated wooden packing material
- Beetle + fungus, single introduction event
- Low genetic diversity (only “good news”) – expected low virulence variability



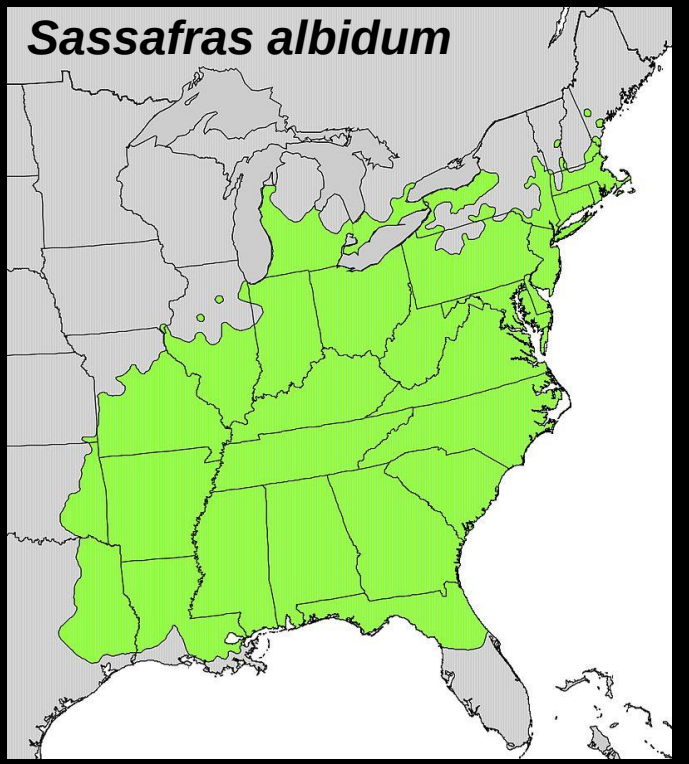
Spread – Long Distance

Current LW distribution (2019)

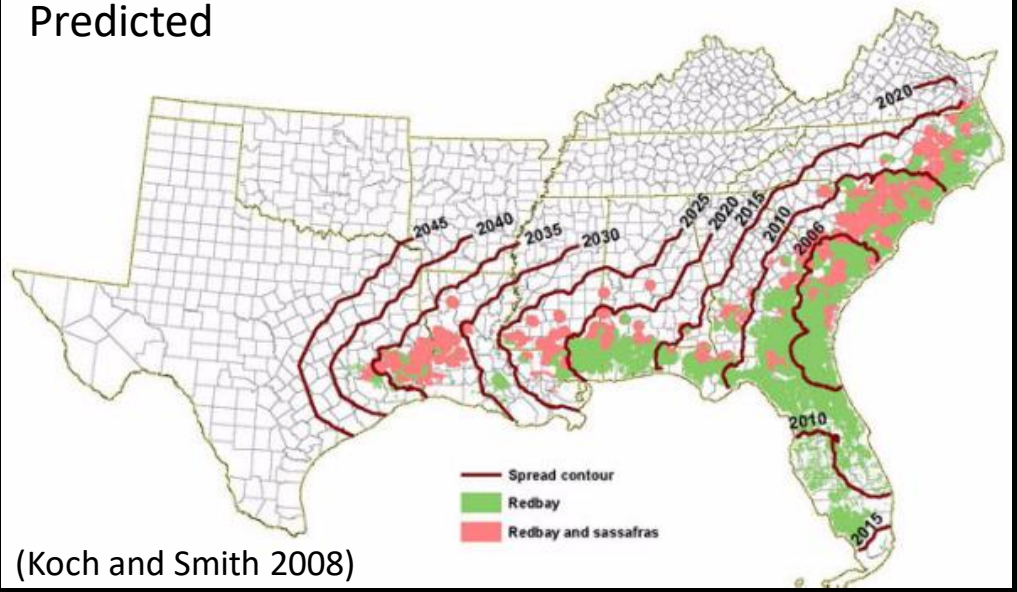


10 States (2019)

Host connectivity allows for rapid-natural-spread



Predicted



(Koch and Smith 2008)

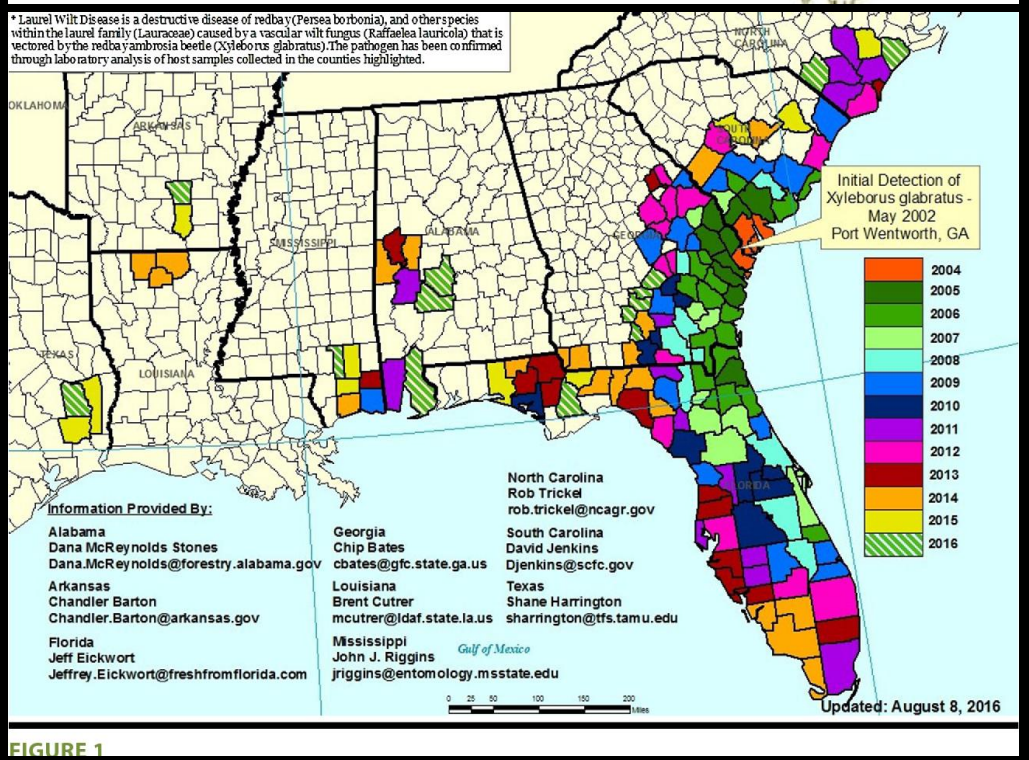
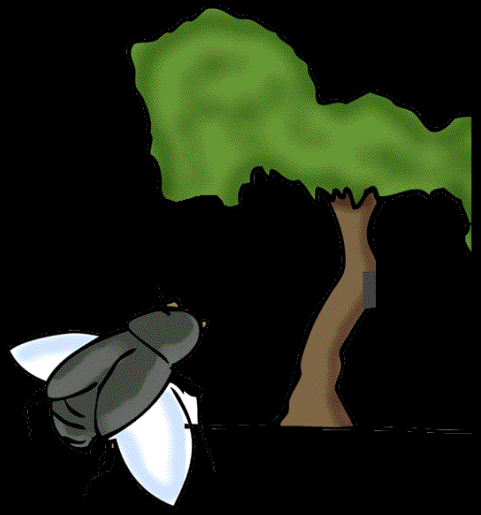


FIGURE 1

Spread Accelerated Through Anthropogenic Movement



<u>Evidence</u>: Comparison between predicted distribution of *R. lauricola* main vector (2006 – 2045) vs. current distribution of the disease (2018)

Predicted arrival to Texas: 2035

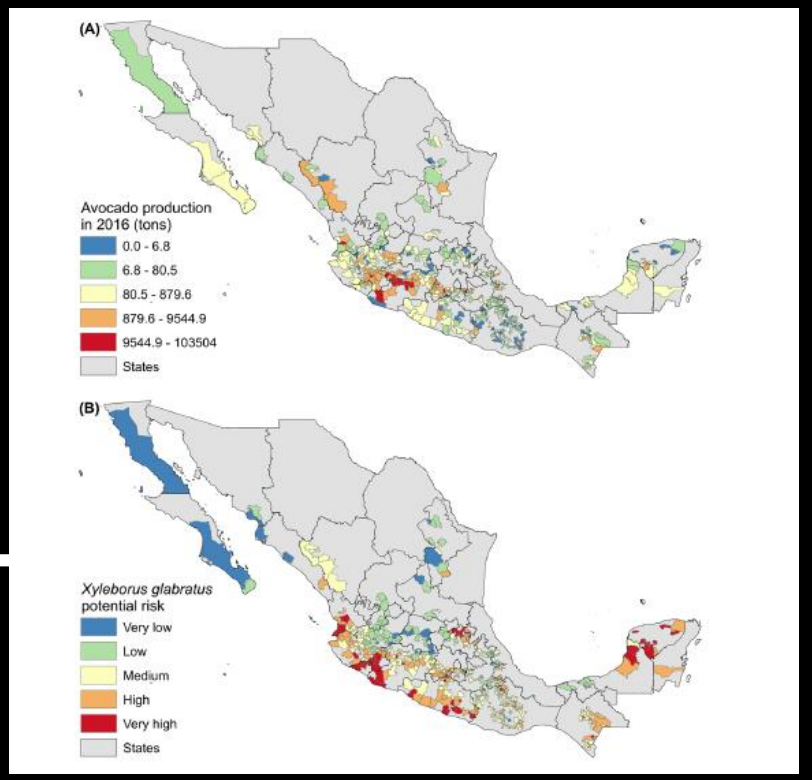
Actual arrival to Texas: 2015

It's Not a Matter of IF but WHEN

Lauraceae is a diverse and widely distributed family with multiple Neotropical species

In addition: Red Ambrosia Beetle (in its native range) also uses other plant families as reproductive host

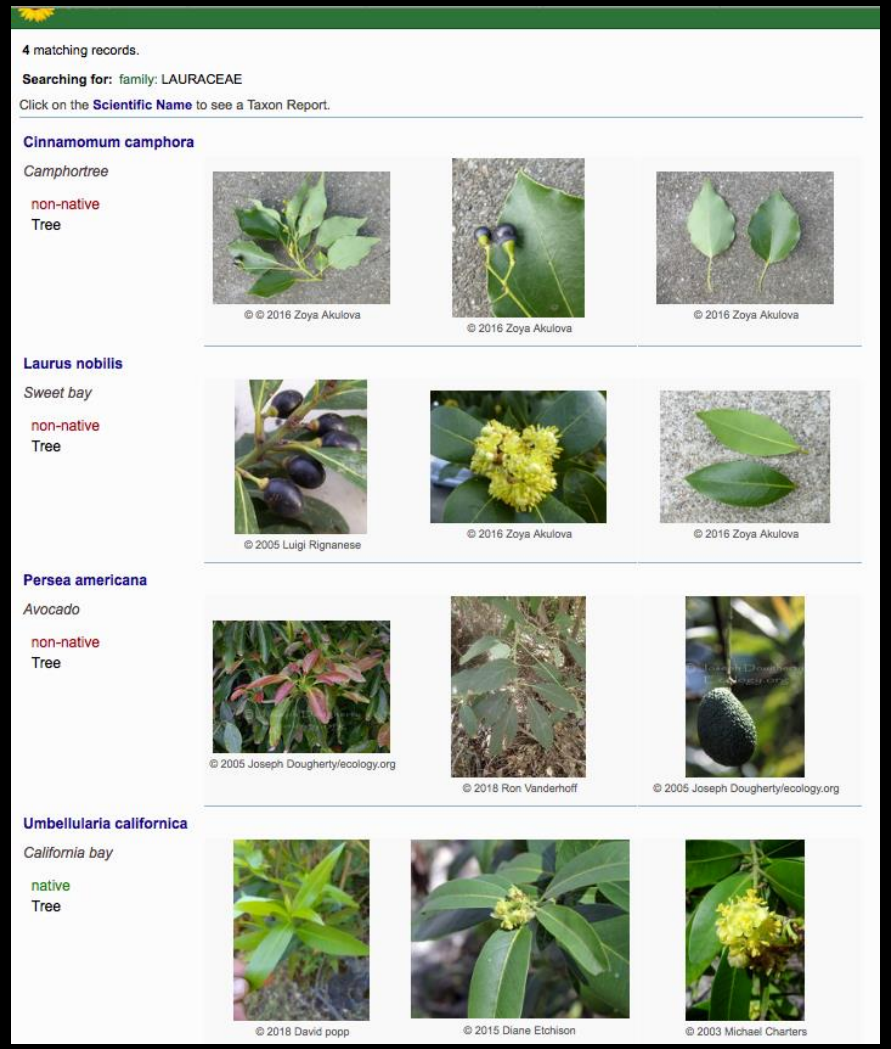
Potential invasion of exotic ambrosia beetles *Xyleborus glabratus* …in Mexico
Lira-Noriega et al. 2018.*Scientific reports*, *8*(1), 10179.

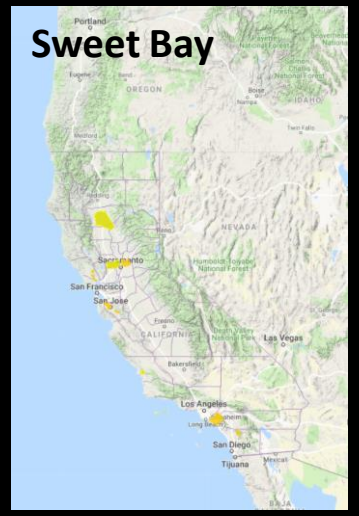


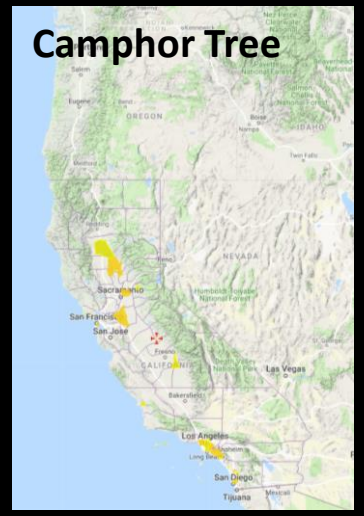
Risk to California Avocado Industry

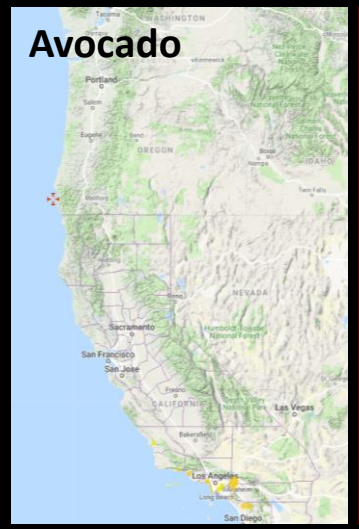
Could a LW outbreak happen in CA?

- Host Susceptible (fungus - YES) /reproductive host (beetle)?
- Conducive environment (climate) YES
- Introduction pathway (natural/anthropogenic) ?





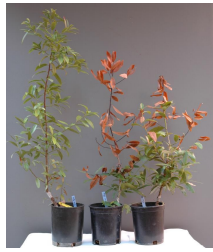


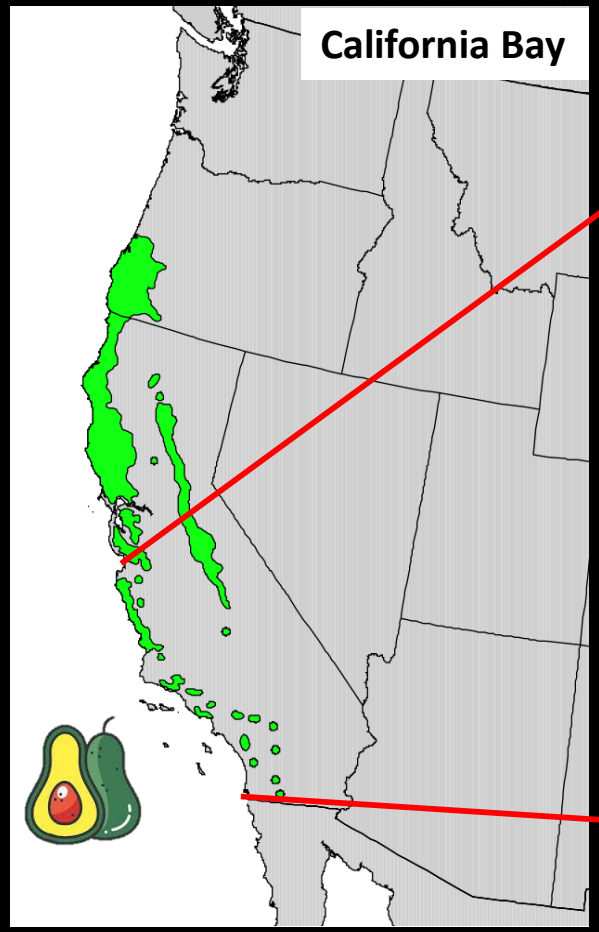


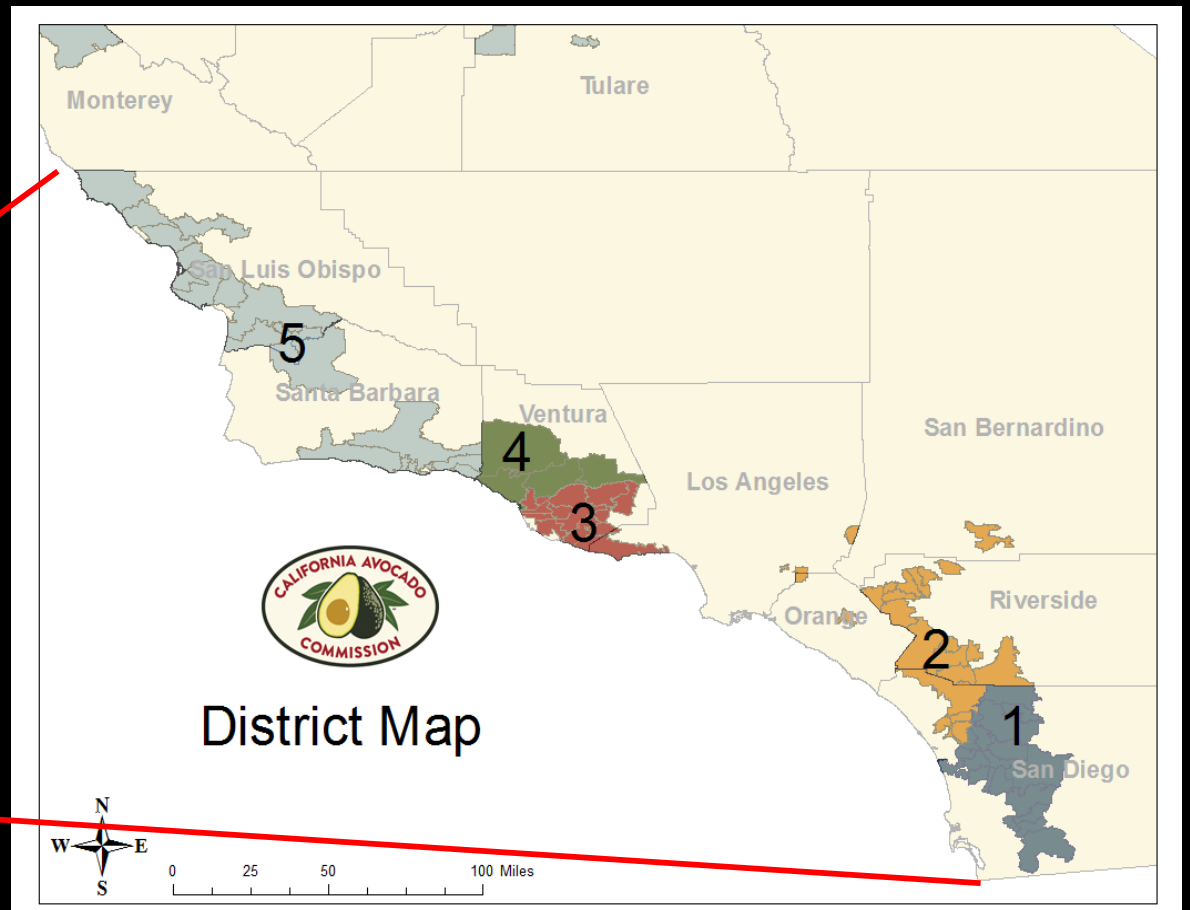
Fraedrich, S. W. *Plant Disease* 92, no. 10 (2008): 1469-1469.

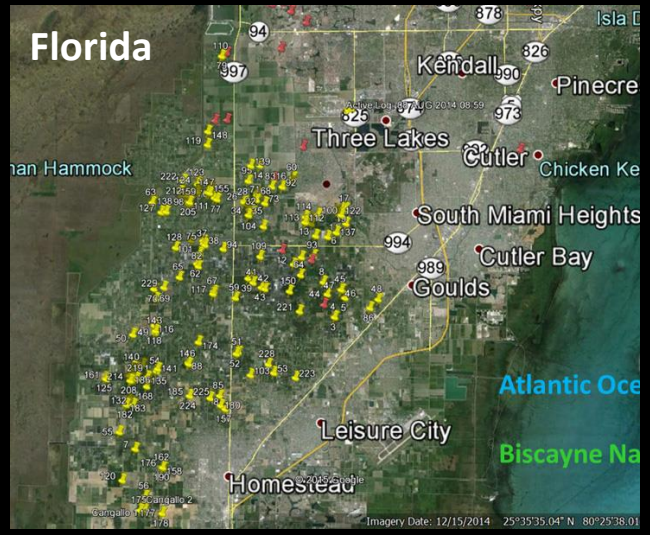
Risk to California Avocado Industry

Could a LW outbreak happen in California?



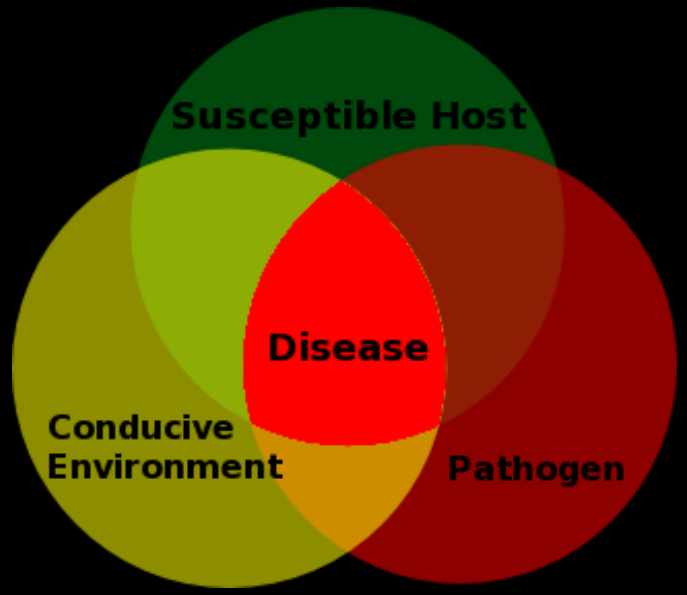






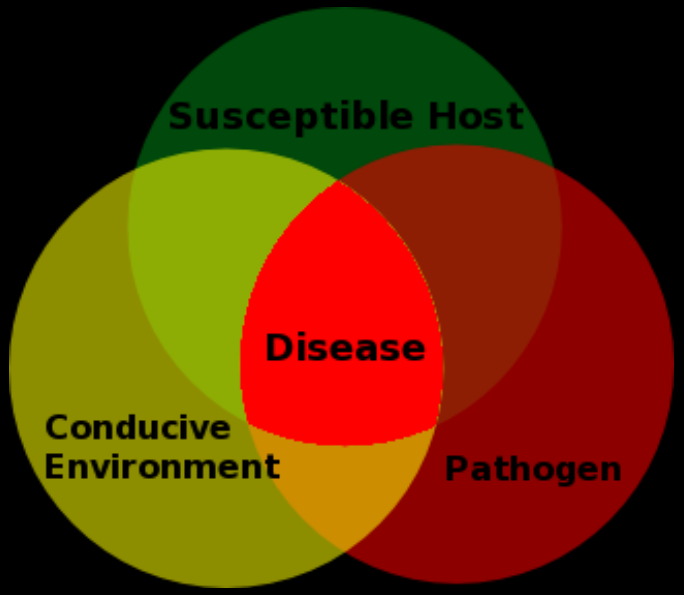
<u>It will depend on multiple factors</u> : Are the wild susceptible hosts (California bay) distributed close to the avocado groves? How dense do these tree grow; potential lateral transfer of the fungus to local bark beetles that are attracted to avocado; likelihood avocado trees are root grafted; among other….

MANAGEMENT: IT'S COMPLICATED



- Very virulent strain
- Multiple susceptible hosts
- Wild susceptible hosts
- Multiple efficient vectors
- Two transmission modes (insect/root-to-root)

MANAGEMENT: IT'S COMPLICATED



- Very virulent strain
- Multiple susceptible hosts
- Wild susceptible hosts
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- Two transmission modes (insect/root-to-root)

Research has been VERY challenging due to the numerous variables that can influence the experiments: age, cultivar, environment, previous management, etc. Therefore a large number of trees are needed.

Strategies we have tried….

- Fungicides
- Biological Control (endophytes)
- Stump-bag heat treatment
- Sanitation and Tree disposal (Dr. Crane)
- Trenching
- Insecticides

1. Multiple fungicide active ingredients were tested *In Vitro.* **Triazoles and** (Fluazinam*, Azoxystrobin, Pyraclostrobin, Fluoxastrobin*) **had high activity against RL**

2. Only triazoles were effective in greenhouse experiments, Propiconazol showed the highest activity

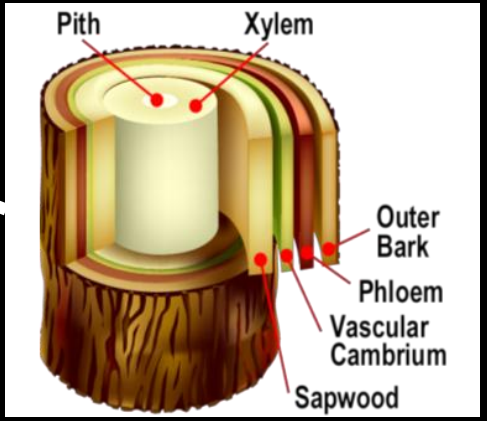
3. Propiconazol was tested in different formulations and delivery strategies in greenhouse experiments. Drench and bark applications were not effective

Field Testing on productive trees

More Resources

More Variables

Product needs to be delivered into the xylem

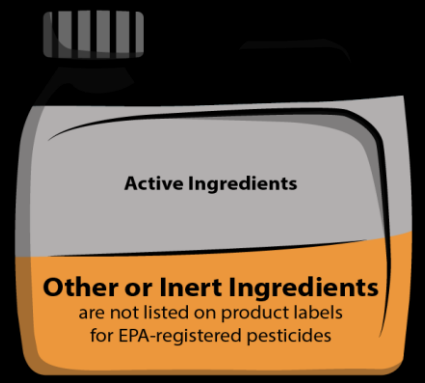


- Propiconazole is a systemic foliar fungicide with a broad range of activity and use in multiple crops
- Propiconazole interferes with sterols production which is critical to the formation of cell walls of fungi
- Therefore, propiconazole is considered to be **fungistatic or growth inhibiting rather than fungicidal or killing**

FUNGICIDE RESEARCH Greenhouse and Field Experiments

Wish list:

- Formulation that can be applied to the tree's vascular system (vascular pathogen) but can be used in FOOD CROPS. Several formulations in the market targeting landscape trees.
- Formulation that assures a systemic delivery of the active ingredient, evenly through out the tree. Solubility is important!
- Formulation that preserves the active ingredient (longevity of the product within the tree)



NOT Tested

Tested

14.3% A.I.

Injectable formulations

Not registered for fruit bearing trees

41.8% A.I.

Registered for food crops

38.70% A.I.

Registered for food crops

FUNGICIDE RESEARCH – Delivery System for Field-grown Trees

Macroinfusion: high volumes of dilute fungicide into root flares

Microinjection: application of low volumes of concentrated fungicide

Fungicide Efficacy Trial

Riley #2, 23 treatments

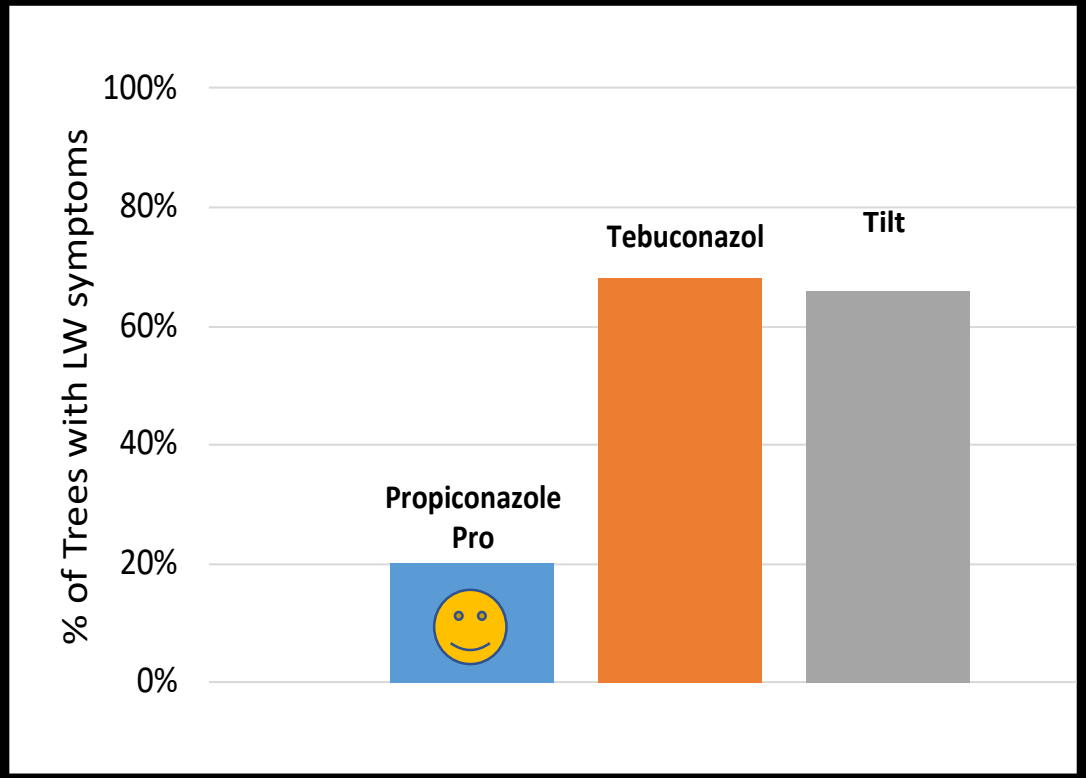
Trees inoculated **42 days after fungicide application**

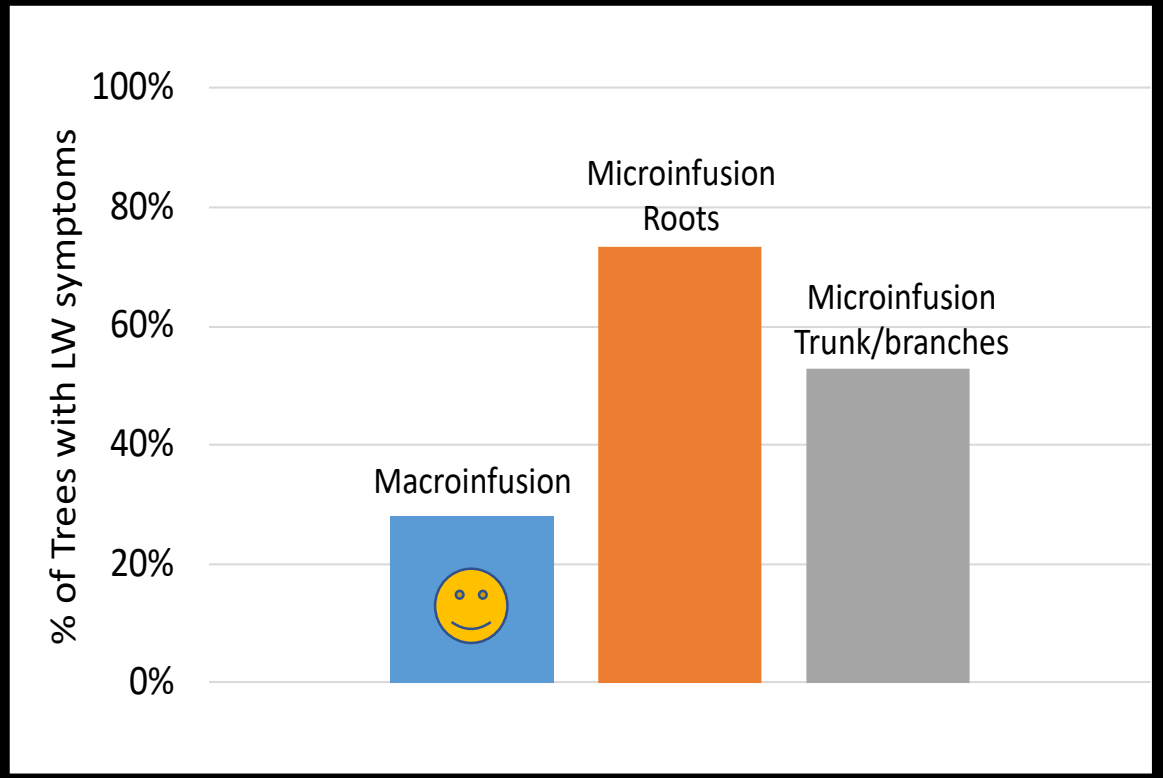
Rating @ 4 months. 5 reps/treatment

Overall:

Propiconazol Pro performed the best

The best method was Macroinfusion





Fungicide Efficacy Trial

Riley #2, 23 treatments

Trees inoculated **42 days after fungicide application**

Still 20% of the trees get LW

Remember….pathogen spreads rapidly from one infected tree

Propiconazol Pro NOT LEGAL to use in food crops

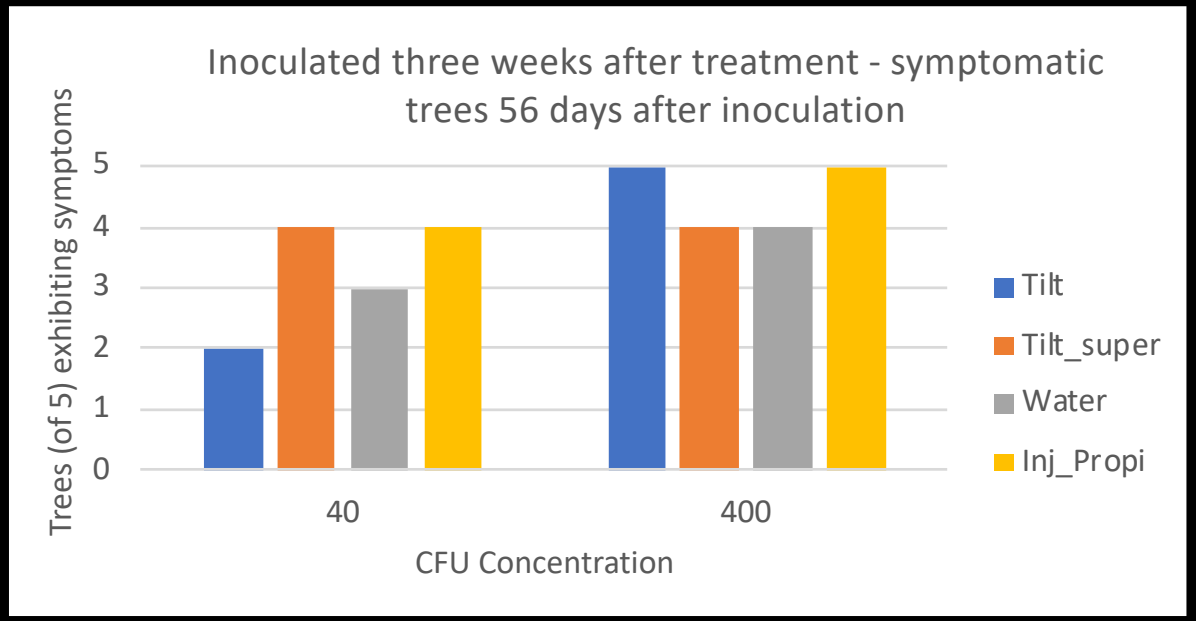
Macroinfusion is too expensive

Can we improve efficacy by injecting the product earlier? (allow time for the A.I. to distribute within the tree)

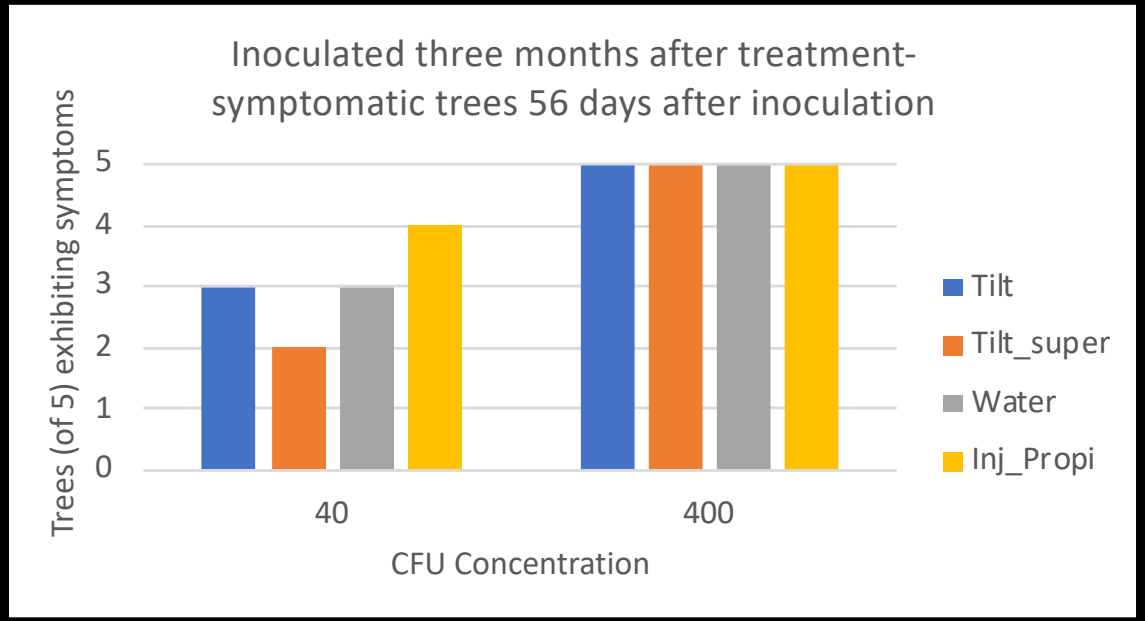
1) Tilt standard mixture – microinjection
2) Centrifuged Tilt – microinjection
3) Propiconazole Pro – microinjection

Inoculum

40 spores vs. 400 spores



3 weeks before, rating 56 days after inoculation



3 months before, rating 56 days after inoculation

- *Not enough trees* (5 trees per treatment)
- Products performed worse or the same when injected 3 weeks vs. 3 months before inoculation (low inoculum)
- More research is needed – More replicates, longer timeline

FUNGICIDE RESEARCH – some issues

- Uneven distribution of the product – pockets of high [ ] but multiple areas of low [ ]
- Infected trees must be re-treated every year $$$
- Fungicide injection damages trees (report from growers)
- Since the pathogen is not eliminated in treated trees (fungistatic not a fungicide), infected, treated trees are reservoirs for the pathogen and subsequent root graft transmission
- Fungicide treatment alone is not a sustainable solution – but it maybe useful as preventative strategy to “buy” time to apply sanitation (tree removal)

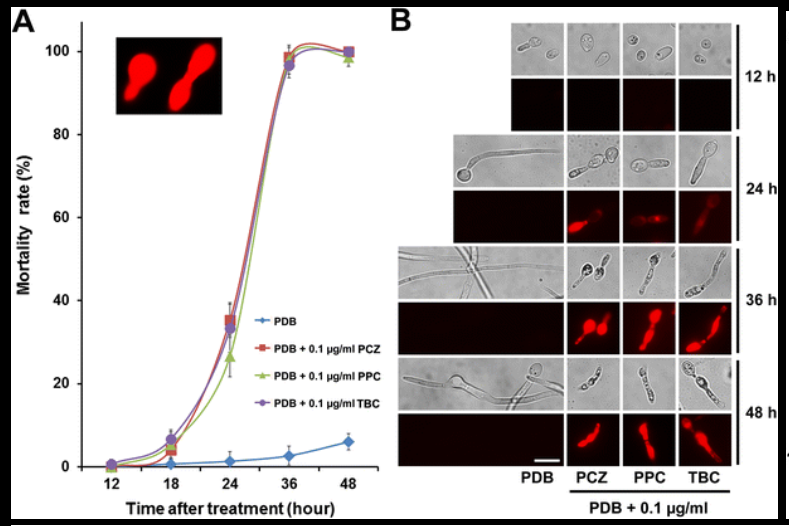
FUNGICIDE RESEARCH – Is There Hope?

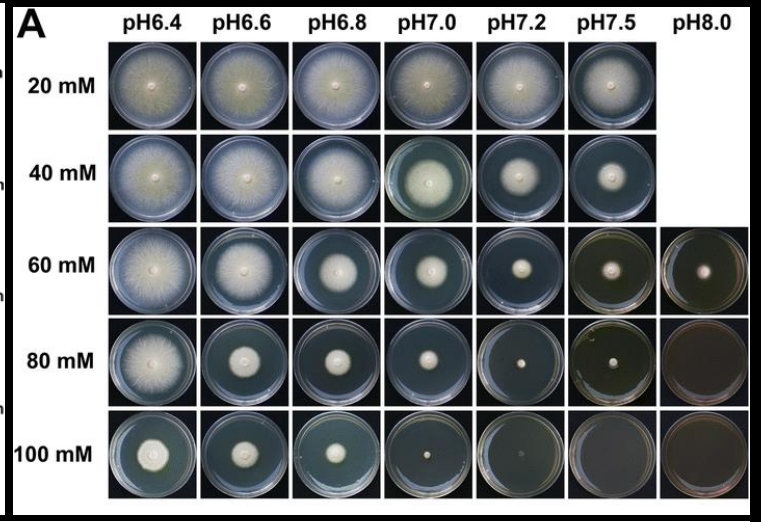
Zhou et al. 2018. Identification of the Achilles heels of the laurel wilt pathogen and its beetle vector. Applied microbiology and biotechnology, 102(13)

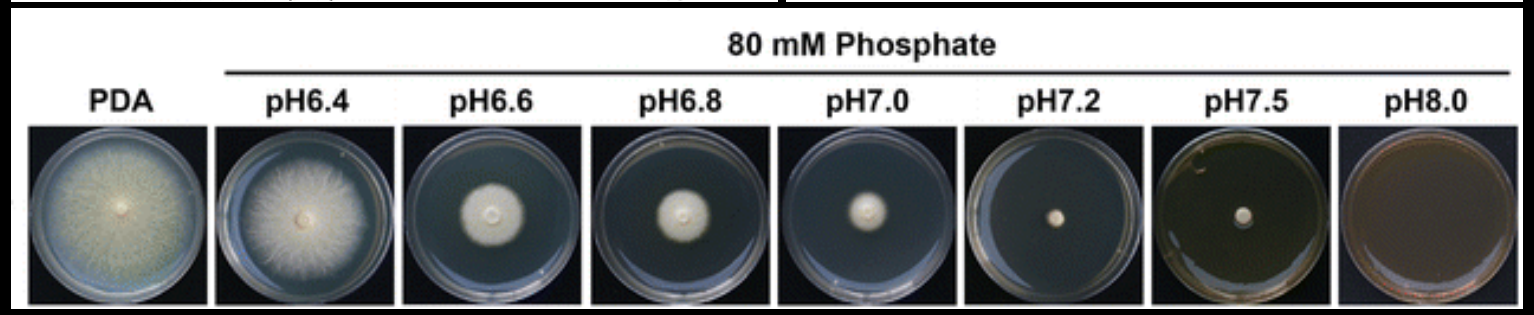
Prochloraz higher *in vitro* activity, pH (higher) and phosphates (higher) could improve efficacy

Table 1
IC_{50} and IC_{95} of the most potent fungicides on th

Fungicides	$IC_{50/95}$/SE	*Raffealea* *R. lauricola*
Prochloraz	IC_{50}	4.5
	SE	0.2
	IC_{95}	6.7
	SE	0.6
Propiconazole	IC_{50}	14.2
	SE	0.8
	IC_{95}	35.8
	SE	1.4
Tebuconnazole	IC_{50}	22.8
	SE	0.9
	IC_{95}	31.6

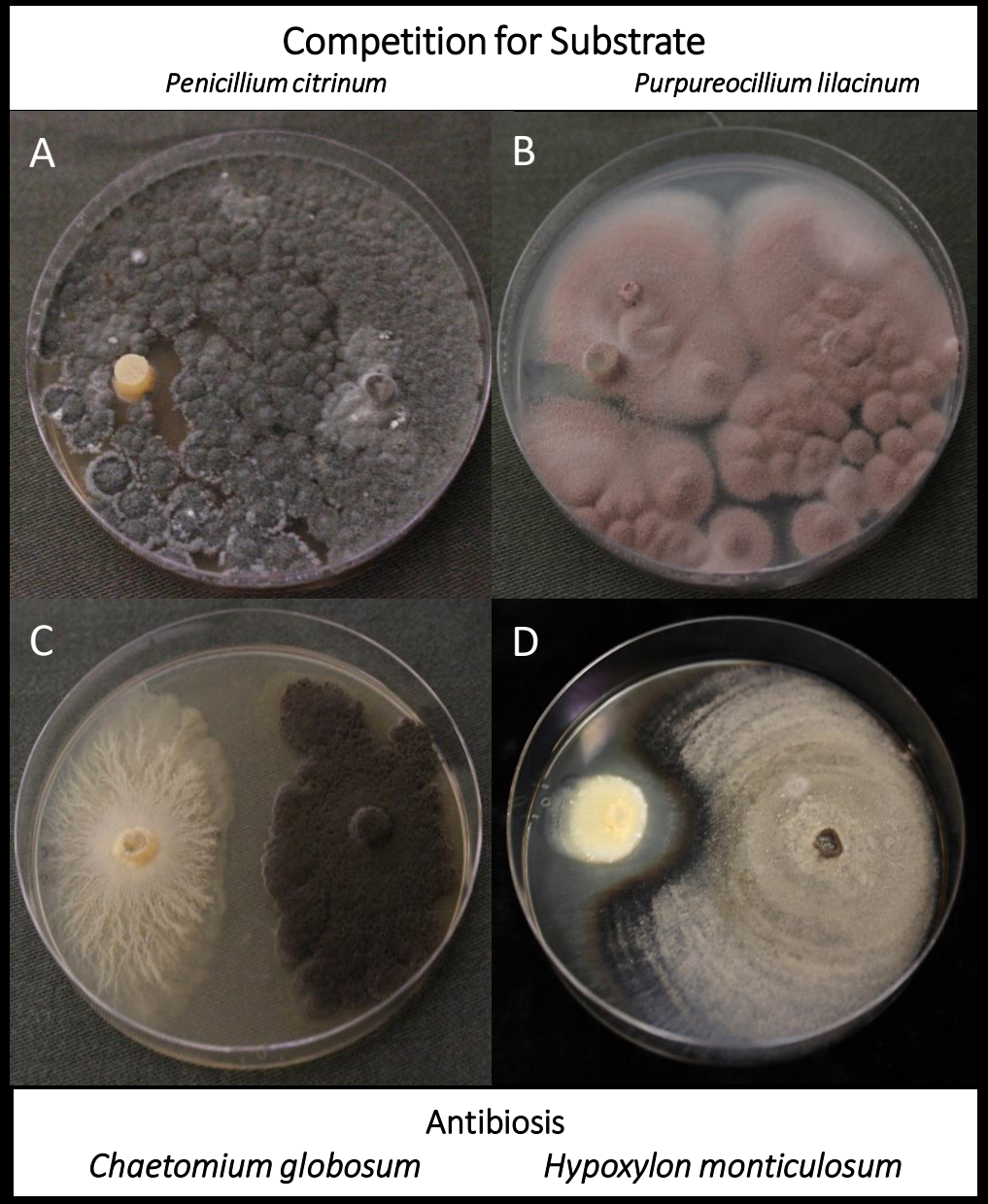






***In vitro* → *In planta?* → Field-grown producing trees?**

Biological Control: Endophytes



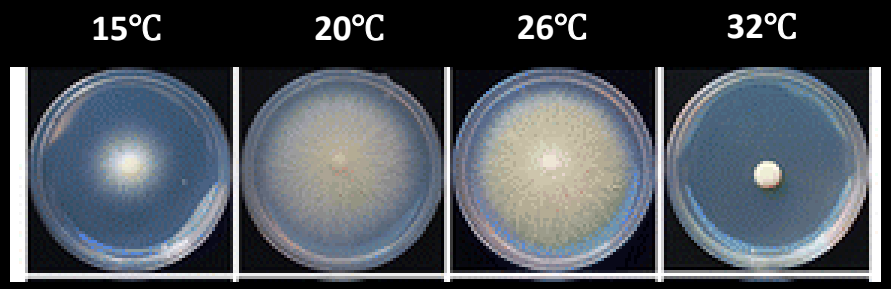
In vitro ~~→~~ ***In planta***

Although the pathogen rapidly and thoroughly colonized test trees, colonization by the tested endophytes was minimal

Pérez-Martínez, J., Ploetz, R.C. and Konkol, J.L., 2018. Significant in vitro antagonism of the laurel wilt pathogen by endophytic fungi from the xylem of avocado does not predict their ability to control the disease. Plant pathology, 67(8), pp.1768-1776.

How about endophytic *Trichoderma* or other entomopathogenic species?

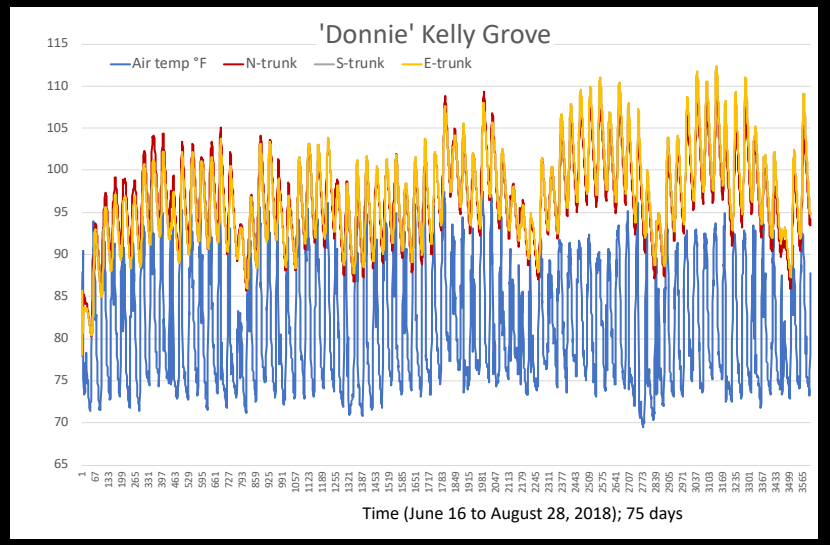
STUMP-BAG HEAT TREATMENT (trees adjacent to trees removed due to LW)



Zhou et al. 2018

Temperature inside the trunk did reach lethal temperatures for RL …..but the pathogen survives in the roots!

Use of heat to disinfest the trunk from the fungus



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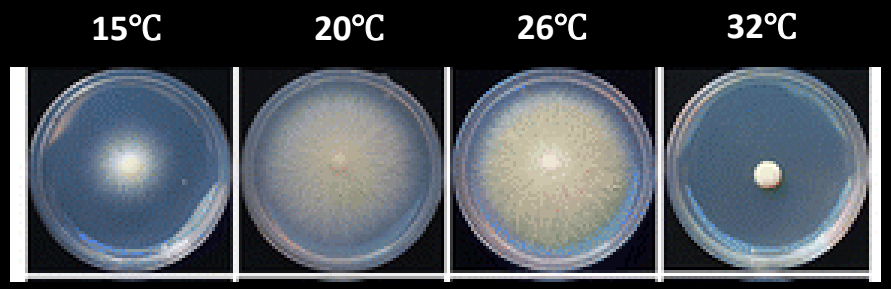

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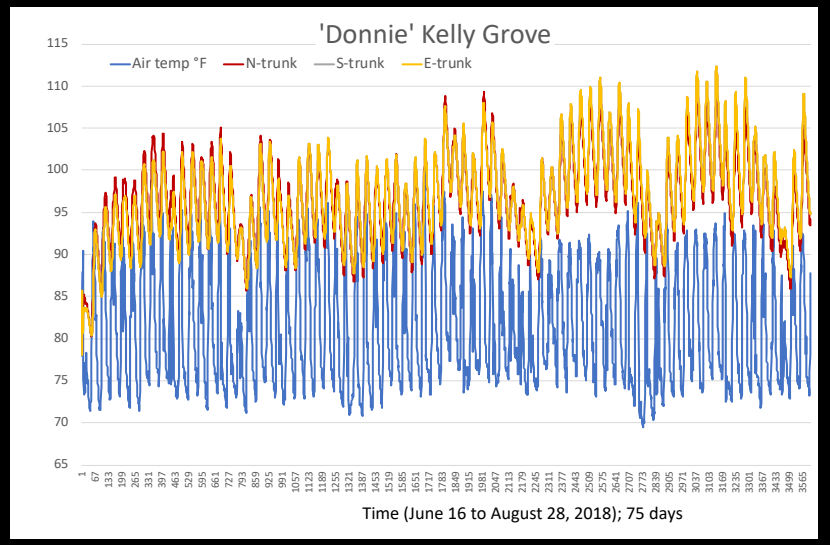
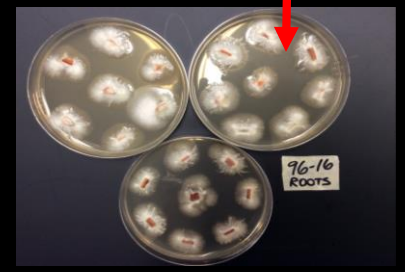


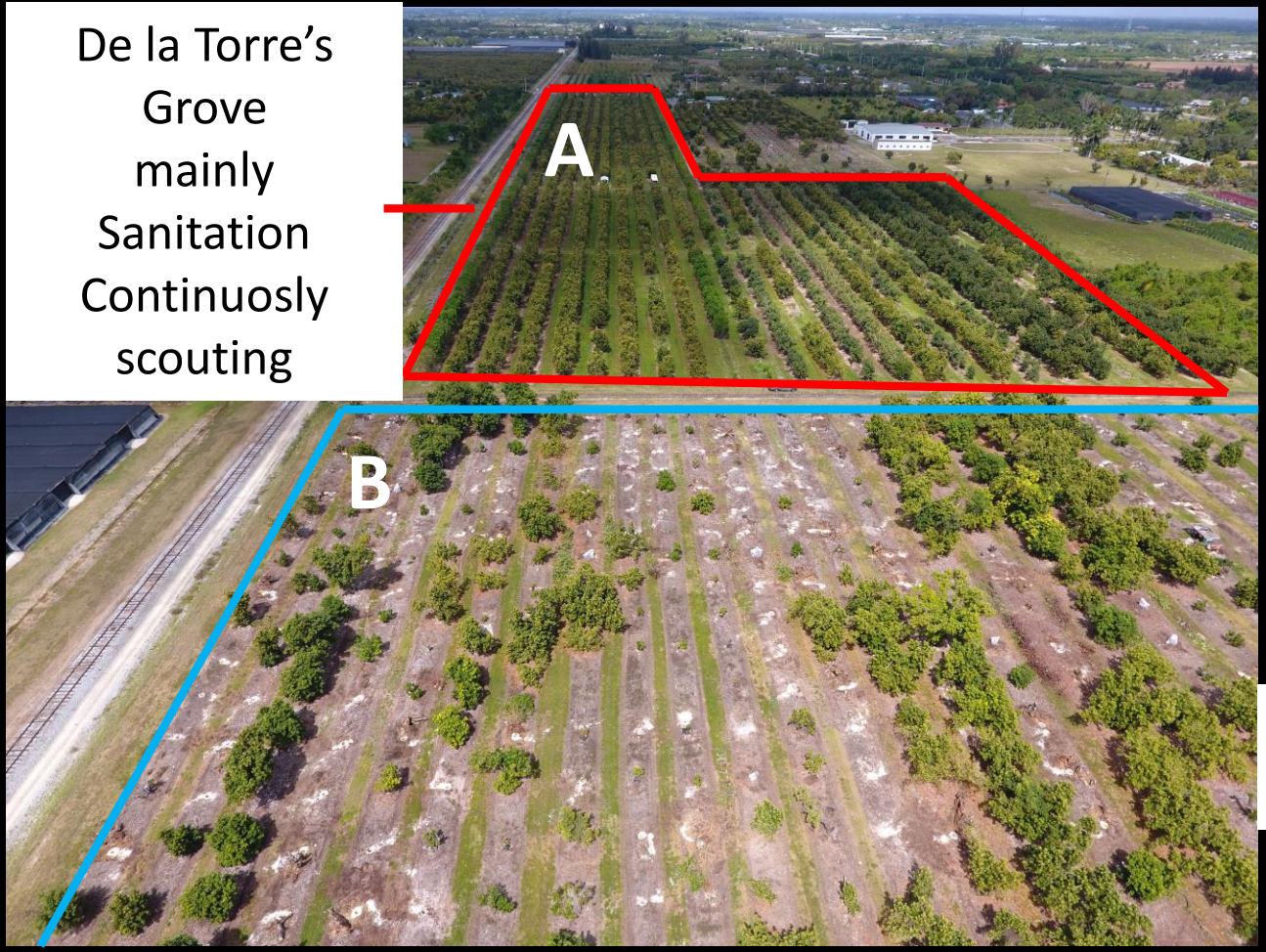


Different results after the “Stump-bag heat treatment” (all trees were infected with LW)

Laurel wilt management depends on <u>prompt and rigorous sanitation</u>

Once laurel wilt is established in an orchard it becomes a much more difficult problem

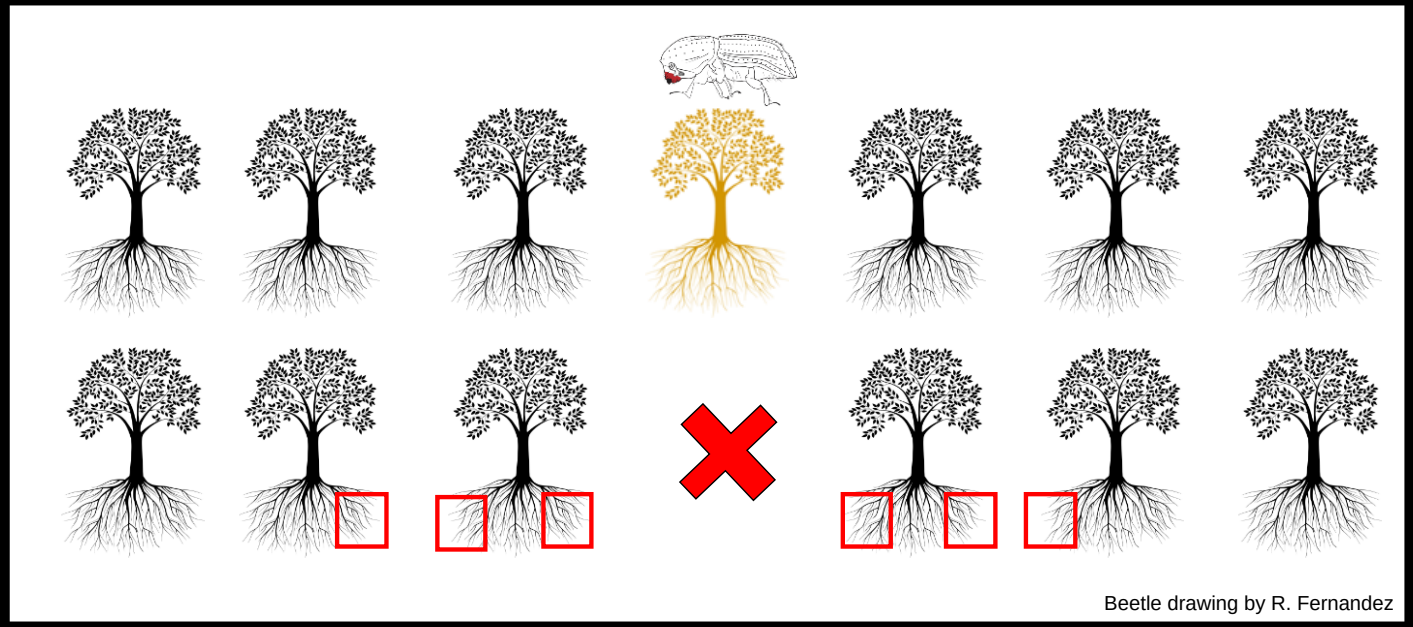
De la Torre's Grove mainly Sanitation Continuosly scouting



Neighbors' Grove
Stumping, Bagging, Injections

Timely detection is CRUCIAL

EARLY AND RAPID DETECTION IS CRUCIAL



How quickly can we detect the pathogen?

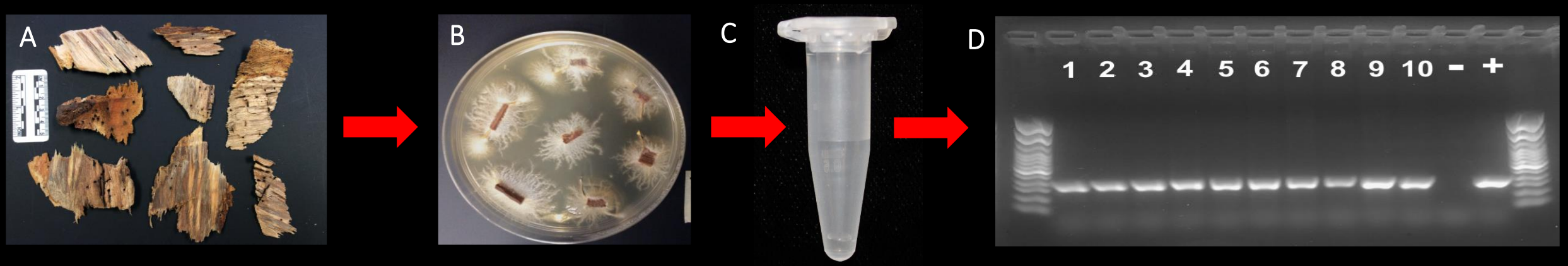
How many trees do we need to remove to contain the spread?

Need a detection method able to detect the fungus in asymptomatic trees

Internal symptoms appear BEFORE external symptoms

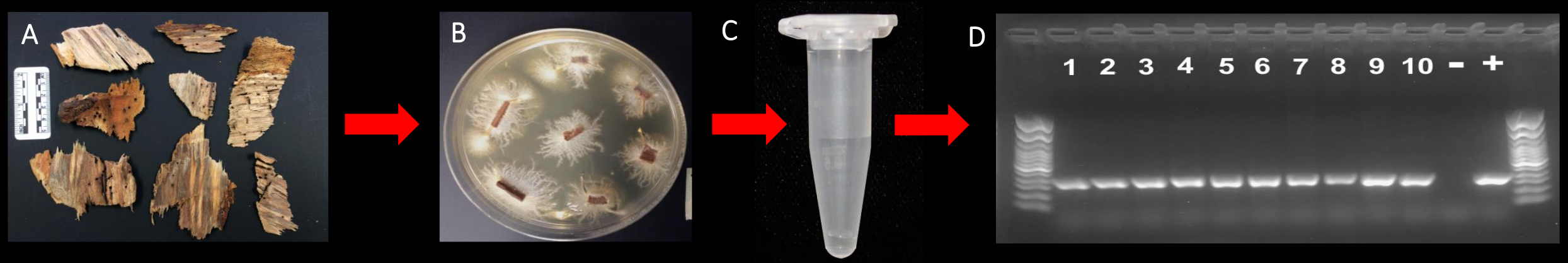
These questions will be addressed by the Forest Service STDP grant (testing will include other *Persea* species and natural forest sampling)

Standard Detection Protocol (trunk tissue from symptomatic trees) = 7-10 days



(A) The infected pieces are plated in the semi-selective media cycloheximide-amended medium (CSMA); (B) fungal growth is observed after 7-10 days; (C) DNA is extracted from the fungal colonies and two primer sets are tested IFW and CHK

Standard Detection Protocol (trunk tissue from symptomatic trees) = 7-10 days



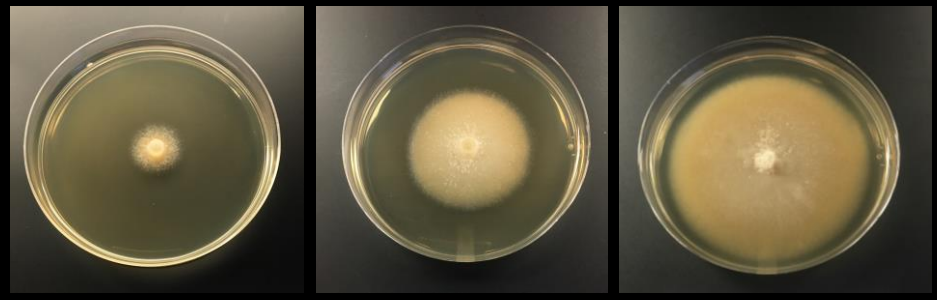
(A) The infected pieces are plated in the semi-selective media cycloheximide-amended medium (CSMA); (B) fungal growth is observed after 7-10 days; (C) DNA is extracted from the fungal colonies and two primer sets are tested IFW and CHK

Raffaelea lauricola

7 days **14 days** **21 days**

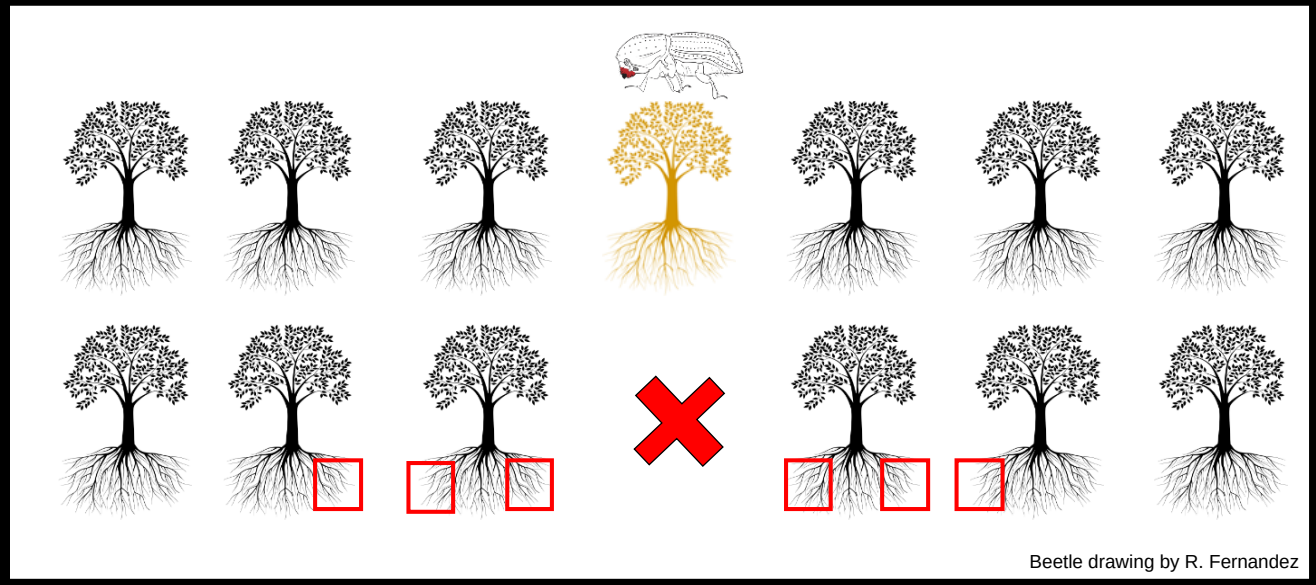
Morphology is not enough to distinguish RL from other *Raffaelea* species associated to avocado (4)

Raffaelea aguacate



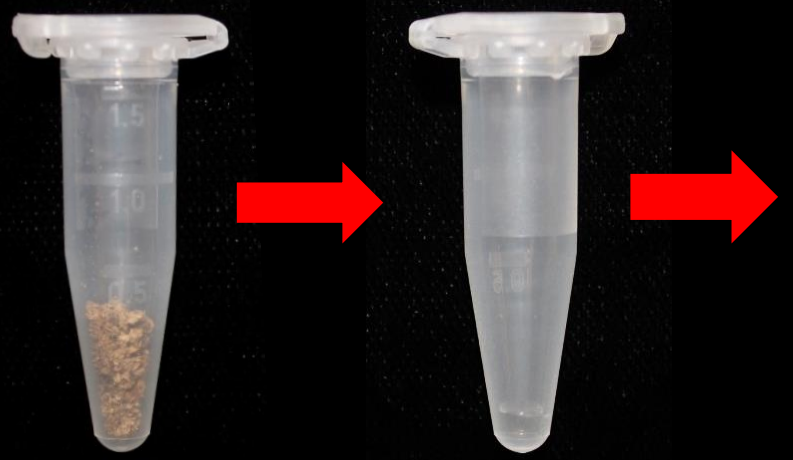
7 days **14 days** **21 days**

Early and Rapid Detection Protocol (root tissue from asymptomatic trees) = 1-2 days



A

B



C

This method could also be applied to other type of environmental samples such as beetles

Managing Laurel Wilt will be difficult.

Ultimately, it may rely on:

1) **Early detection and sanitation**;

2) The use of tolerant genotypes/cultivars in which disease progress advances slower

3) Restricting root graft transmission (trenching);

4) Fungicide treatment in certain situations;

5) Insecticides, repellents and attractants for the vector/s;

Buy you time to apply #1?

Thank you for your attention!

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United States Department of Agriculture
National Institute of Food and Agriculture

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