Laurel wilt: A dangerous new disease of avocado in the Western Hemisphere

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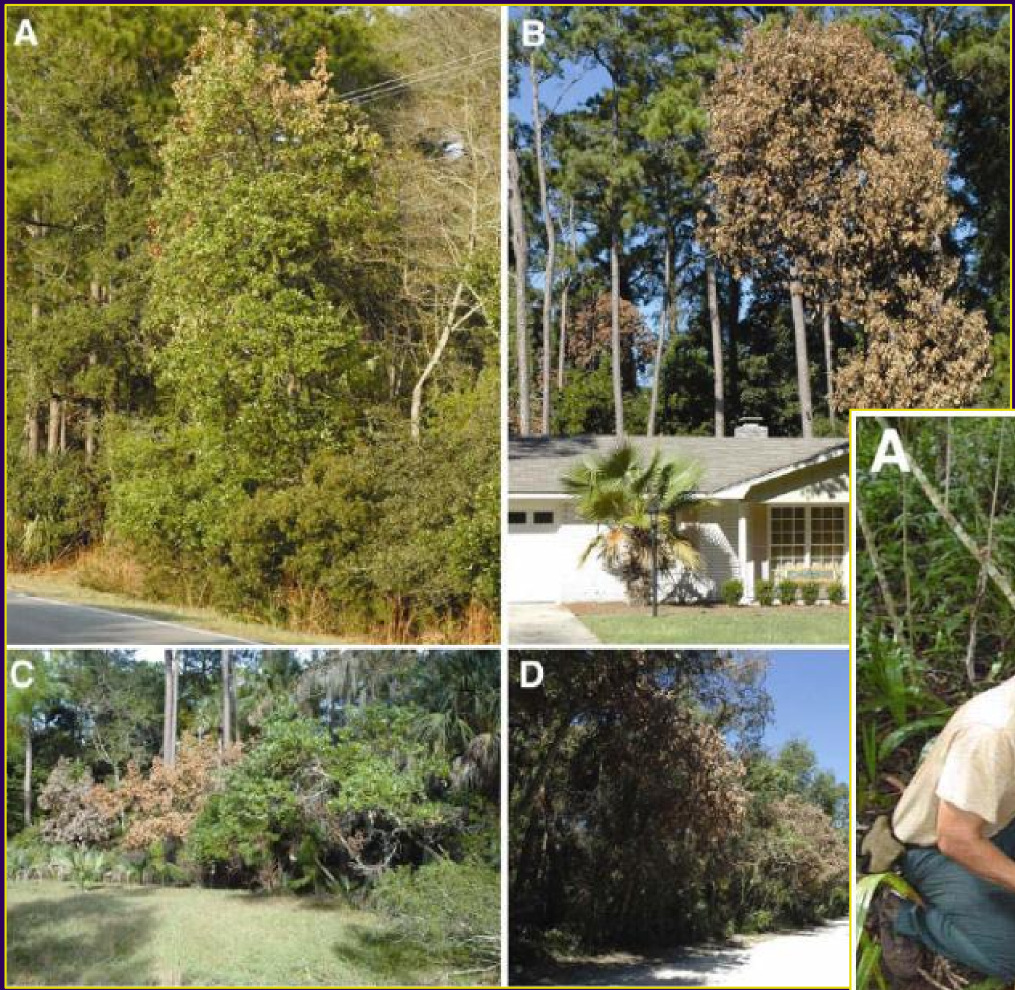
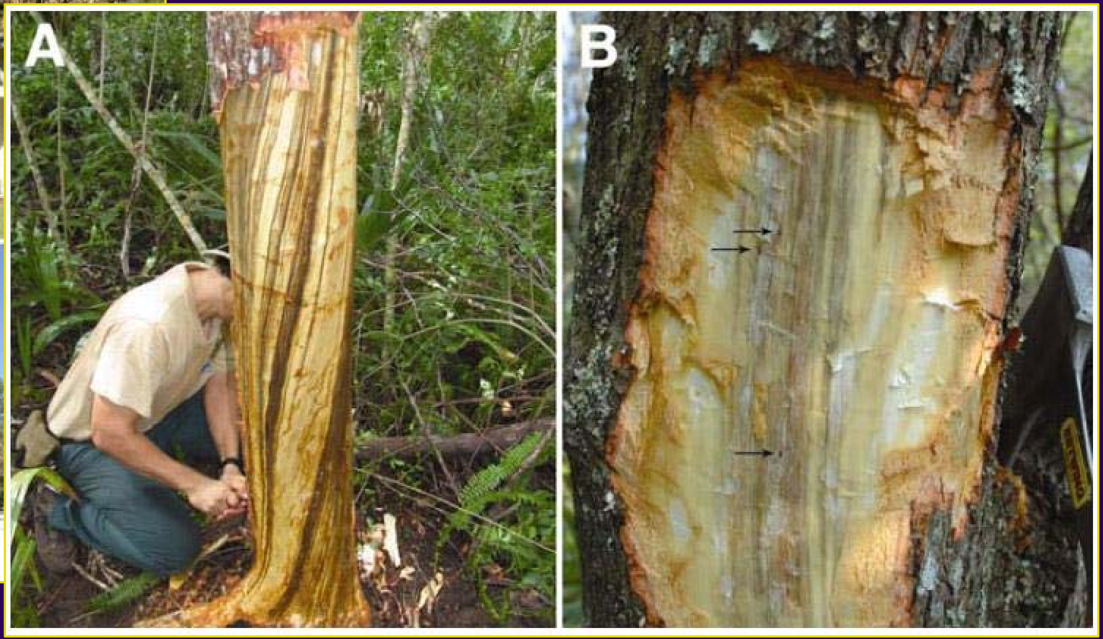
Laurel Wilt Chronology

In 2002, an Asian ambrosia beetle, *Xyleborus glabratus*, was detected for the first time in the Western Hemisphere in Port Wentworth, GA USA

Within a year, red bay (*Persea borbonia*) trees began dying from a new disease, laurel wilt





Fraedrich et al. 2008

In 2006, a new fungus, *Raffaelea* sp., was shown to cause laurel wilt on redbay;

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Raffaelea lauricola, a new ambrosia beetle symbiont and pathogen on the *Lauraceae*

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Abstract — An undescribed species of *Raffaelea* earlier was shown to be the cause of a vascular wilt disease known as laurel wilt, a severe disease on redbay (*Persea borbonia*) and other members of the *Lauraceae* in the Atlantic coastal plains of the southeastern USA. The pathogen is likely native to Asia and probably was introduced to the USA in the mycangia of the exotic redbay ambrosia beetle, *Xyleborus glabratus*. Analyses of rDNA sequences indicate that the pathogen is most closely related to other ambrosia beetle symbionts in the monophyletic genus *Raffaelea* in the *Ophiostomatales*. The asexual genus *Raffaelea* includes *Ophiostoma*-like symbionts of xylem-feeding ambrosia beetles, and the laurel wilt pathogen is named *R. lauricola* sp. nov.

Key words — *Ambrosiella*, *Coleoptera*, *Scolytidae*

Introduction

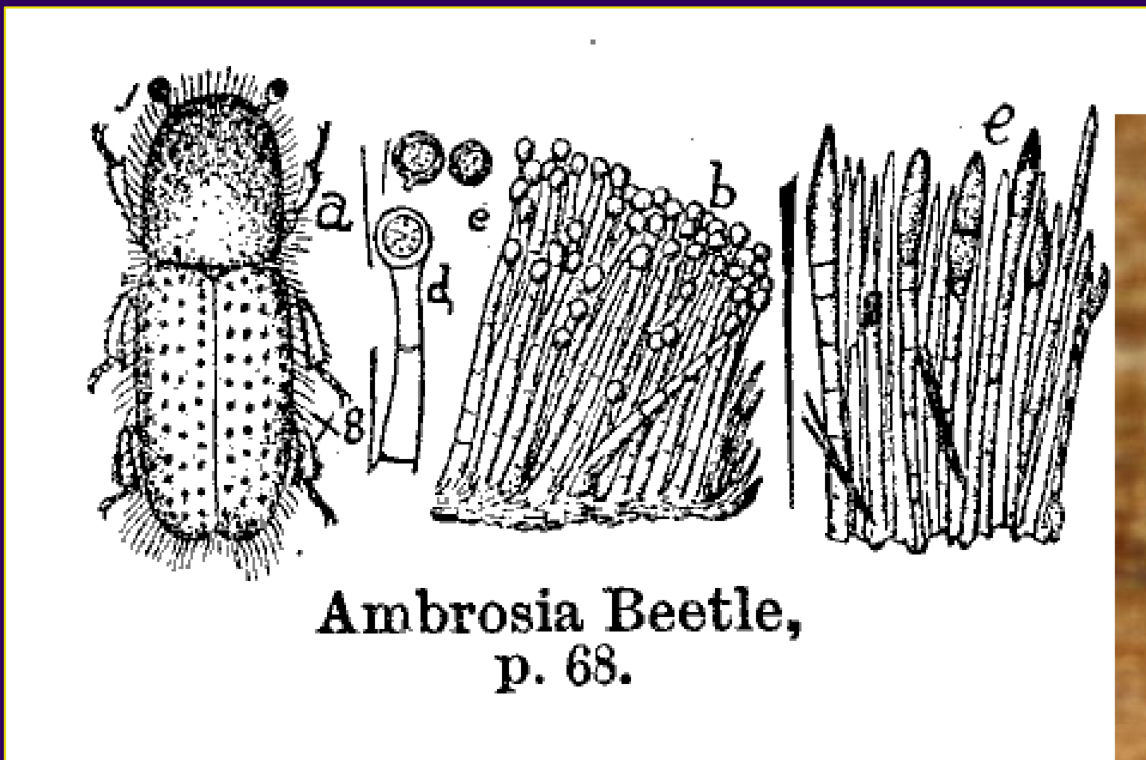
A new vascular wilt pathogen has caused substantial mortality of redbay [*Persea borbonia* (L.) Spreng.] and other members of the *Lauraceae* in the coastal plains of South Carolina, Georgia, and northeastern Florida since 2003 (Fraedrich et al. 2008). The fungus apparently was introduced to the Savannah, Georgia, area on solid wood packing material along with the exotic redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (*Coleoptera: Curculionidae: Scolytinae*), a native of southern Asia (Fraedrich et al. 2008, Rabaglia et al. 2006). As in the case of many ambrosia beetles (Beaver 1989, Harrington 2005), *X. glabratus* has mycangial pouches for carrying fungal symbionts, and the redbay pathogen lives as a budding yeast phase within the mycangium (Fraedrich et al. 2008). Spores of the fungal symbiont ooze out of the mycangium and inoculate the

…it was described as a *Raffaelea lauricola* sp. nov. in 2008…

…and in 2007, *X. glabratus* was shown to vector this new pathogen

Ambrosia beetles are fungus farmers

They consume ambrosial fungi that they cultivate in host trees

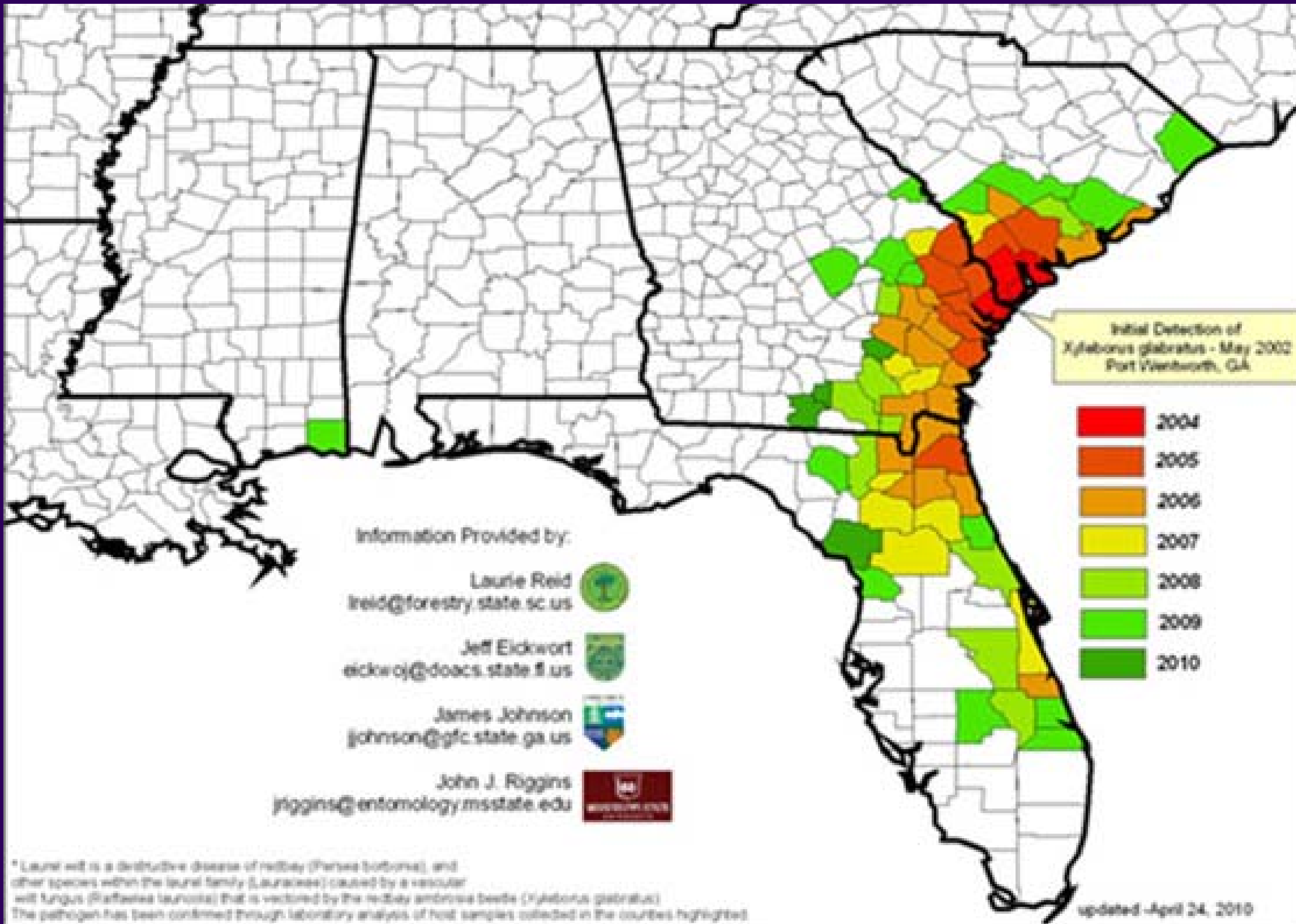


Ambrosia beetle gallery
Robert Rabaglia

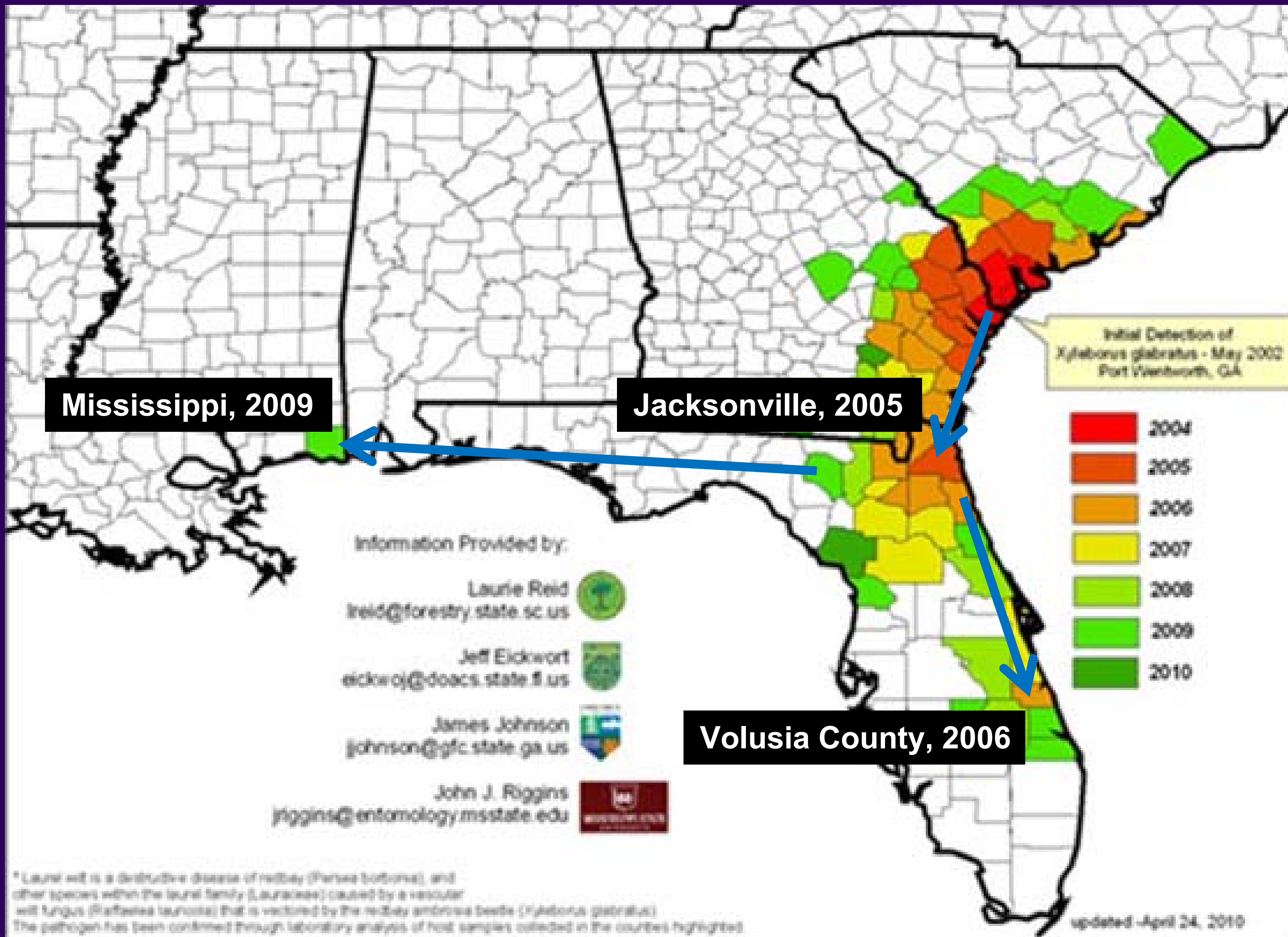
Laurel wilt is an <u>unusual</u> disease

Ambrosial fungi are usually saprobes, but *R. lauricola* is a virulent pathogen

Ambrosia beetles usually affect only dead or stressed trees, but *X. glabratus* attacks healthy trees

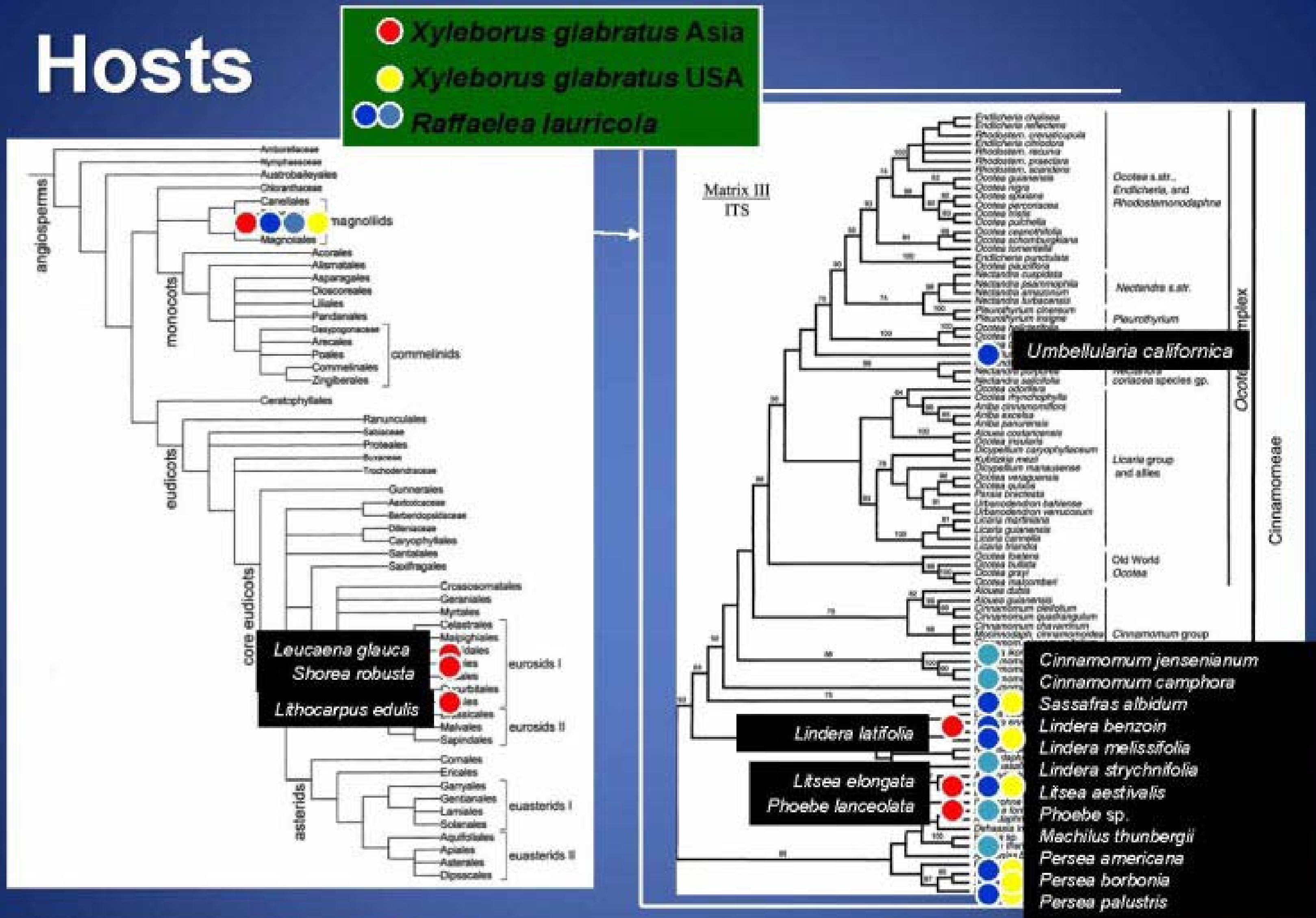


Laurel wilt has moved rapidly
in the southeastern USA



Anthropogenic spread evident/probable causes of long-distance jumps

Hosts



Angiosperms
(http://www.life.uiuc.edu/ib/335/APGII.jpg)

Lauraceae
(Chanderali *et al.*, 2001)

Avocado, *Persea americana*, is the most important agricultural suscept of laurel wilt

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3 botanical races:

- **Mexican (var. *drymifolia*)**
- **Guatemalan (var. *guatemalensis*)**
- **West Indian or lowland (var. *americana*)**

They and their hybrids are cultivated

Avocado, *Persea americana*, is the most important agricultural suscept of laurel wilt

3 botanical races:
- Mexican (var. *drymifolia*)
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- West Indian or lowland (var. *americana*)

They and their hybrids are cultivated

West Indian pedigrees are most important in topical lowlands (Dominican Republic and Florida), Mexican and Guatemalan elsewhere (e.g. California, Mexico)

In 2008, 7 of top 10 producers were in the Western Hemisphere

California and Florida were the top states in USA (respectively, ca. $300 and $50 million annually)

Country	Area under Production (ha)	Total production (metric tonnes)
1. Mexico	114,471	1,124,565
2. Chile	39,842	250,000
3. Indonesia	19,786	225,180
4. Dominican Republic	6,300	187,398
5. Colombia	18,470	183,968
6. Brazil	10,550	166,000
7. Peru	13,603	121,720
8. Spain	15,070	120,000
9. USA	29,473	114,305
10. South Africa	17,000	99,650
Global total	423,624	3,532,011

[a]Figures from FAOSTAT, 2010

Laurel wilt is an immediate threat to:

- **commercial and residential avocado production in Florida**

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•National Germplasm Repository for avocado in Miami (USDA-ARS)

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Major production is at risk throughout the Western Hemisphere

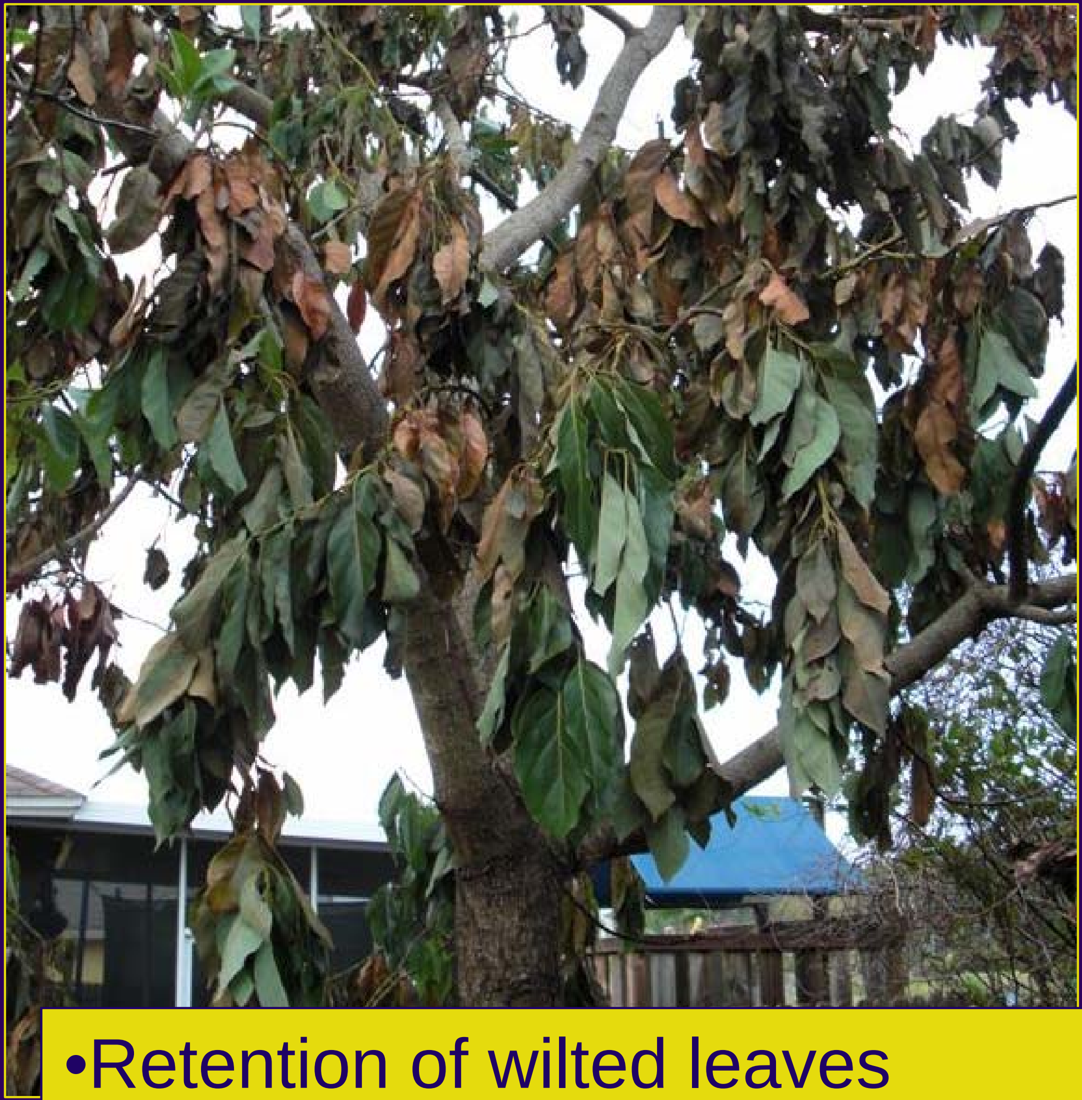
Laurel wilt is an immediate threat to:

- **commercial and residential avocado production in Florida**
- **National Germplasm Repository for avocado in Miami (USDA-ARS)**

Major production is at risk throughout the Western Hemisphere

Global impact??

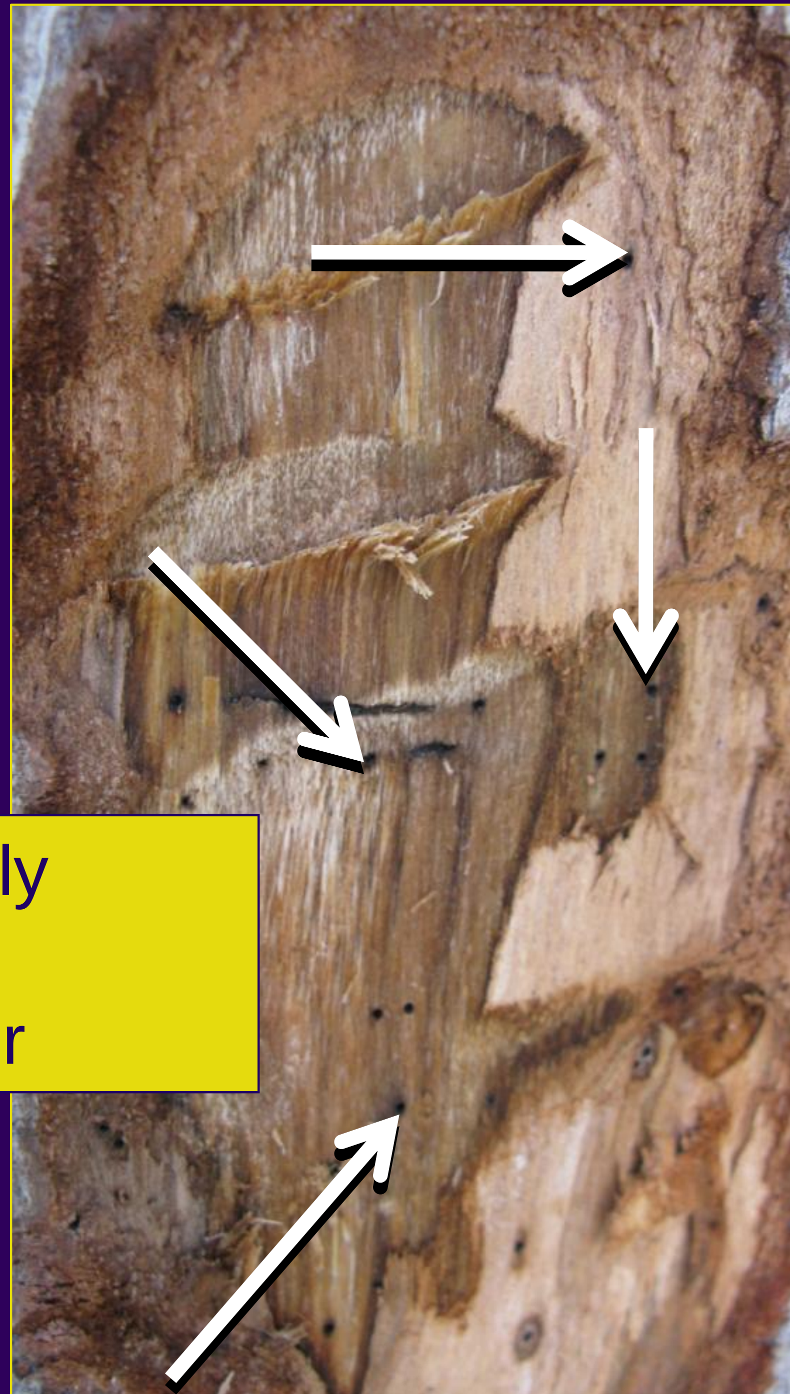
Symptoms



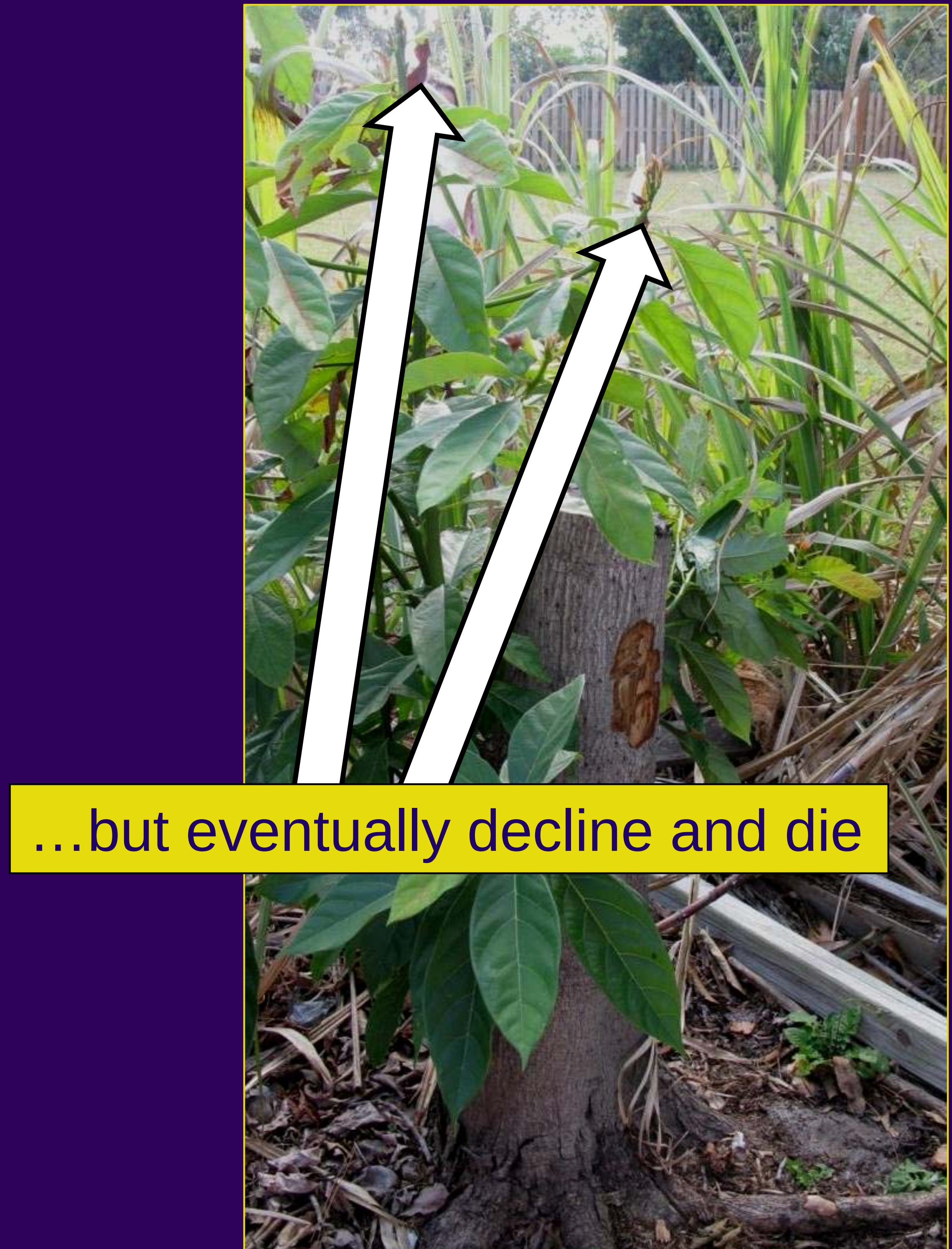
- Retention of wilted leaves
- Sectoral development (in only some traces)

Conspicuous vascular discoloration

…that is eventually associated with evidence of vector activity




Affected trees can re-sprout …


…but eventually decline and die

There are <u>many</u> things we do not know about laurel wilt

•Host range?
•Laurel-wilt resistant avocado?
•Identification and development of tolerant genotypes.
•Resistance mechanisms in avocado and other lauraceous hosts?
•Host x insect x fungus interactions?
•Host or other cues that attract insect?
•Conditions that influence insect's colonization of host plants, completion of life cycle, dissemination to healthy and infected trees (it is unlikely that materials infested with *X. glabratus* have not been shipped to ports other than Port Wentworth)
•Impact of California bay on development and spread of laurel wilt in California?
•Are other magnoliids in ornamental and landscape trades significant hosts for *X. glabratus* and *R. lauricola*?
•Epidemiology of laurel wilt in agricultural and natural ecosystems?
•Efficacy of existing or proposed control measures?
•Economic impact and cost-effectiveness of control measures?
•How should laurel wilt be regulated, interdicted and managed?

Laurel-wilt resistant avocado?

Disease studies

Variables

- **Inoculation method**

Mycelia

Conidia

Disease studies

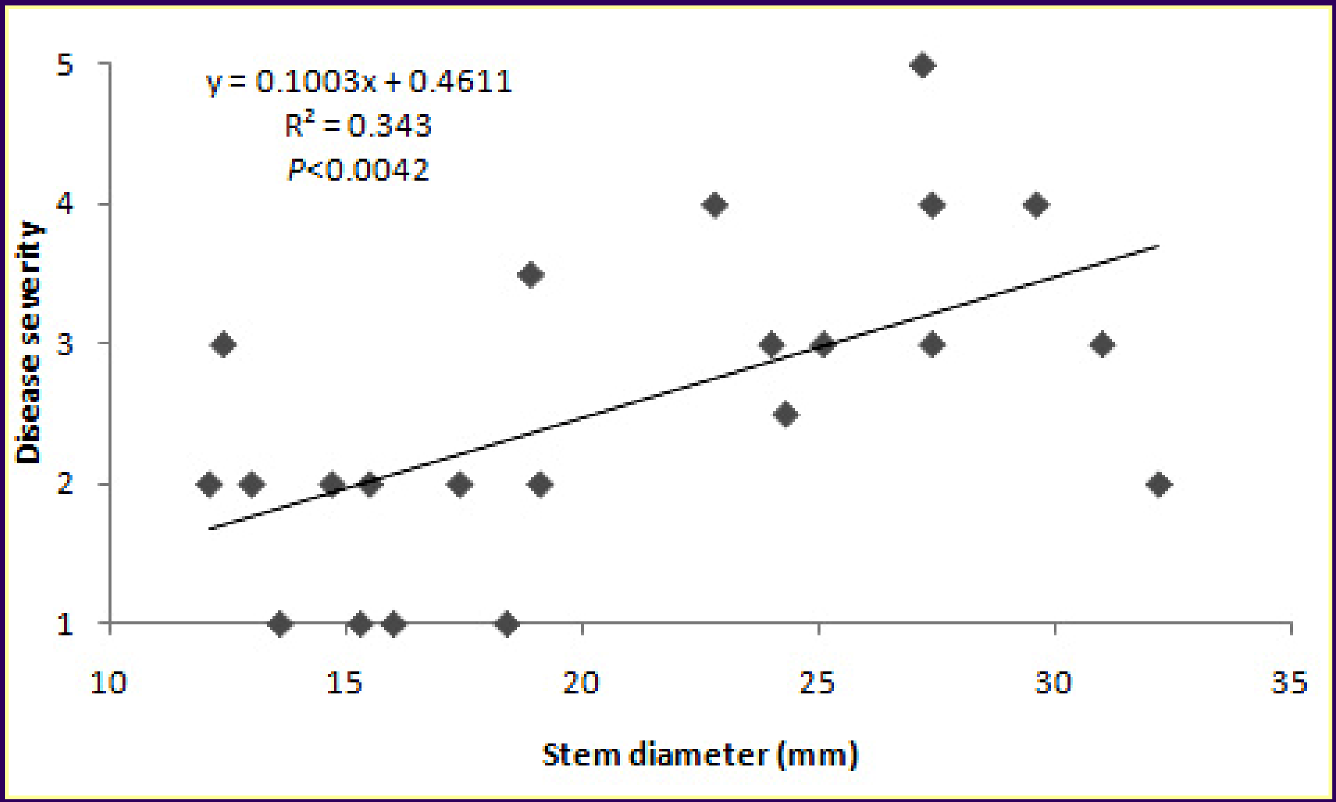
Variables

- **Inoculation method**
- **Isolate variability.** **Available genetic and pathogenicity data indicate founder effect (single introduction) of an asexual fungus – single-spored, stored isolates used in all studies**

Disease studies

Variables

- Inoculation method
- Isolate variability
- **Plant size.** Significant impact



Disease studies

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- Inoculation method
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Disease studies

Variables

- Inoculation method
- Isolate variability
- Plant size. Significant impact – could small plants be used if higher doses of inoculum were used?

Cultivar	Mean disease severity 1 inoc pts		5 inoc pts
Choquette (GxWI)	2.3	ns	3.0
Donnie (WI)	1.3	ns	2.0
Hass (GxM)	1.3	ns	1.3
Lula (GxWI)	1.3	ns	1.7
Monroe (GxWI)	1.3	ns	1.7
Simmonds (WI)	2.3	ns	2.0

Requirement for large plants complicates screening: large plants expensive, not available for many cvs

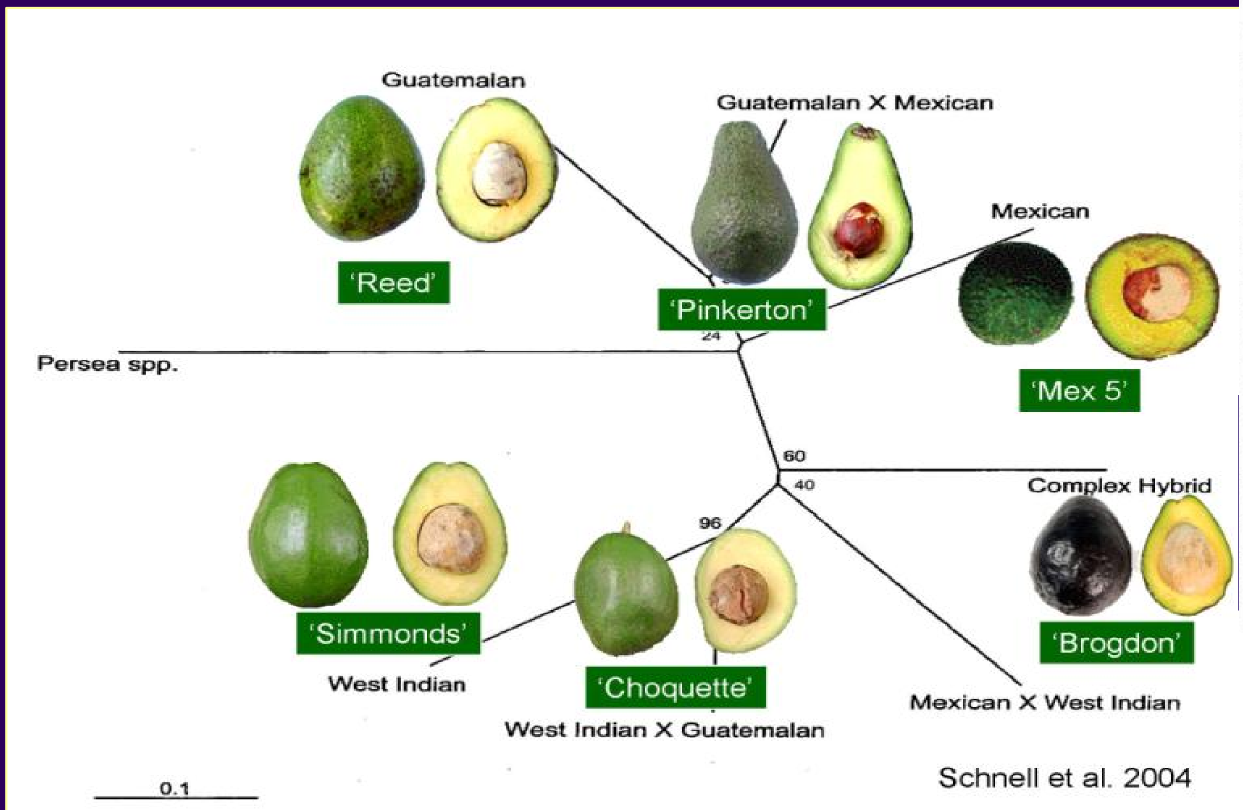
Disease studies

Variables

- Inoculation method
- Isolate variability
- Plant size
- Cultivar

Cultivar screening

Replicated field trials in 2008, 2009 ,2010, and planned for 2011



	Rows																			
Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Cell
NN				9a	18a	6b	13b	12c	5c	1d	12d	15e	7e	3f	2f	12g			10h	NN
MM				8a	17a	18b	11b	10c	14c	13d	4d	8e	2e	17f	13f	4g	9g	8h	9h	MM
LL				7a	16a	5b	1b	6c	8c	10d	3d	16e	12e	4f	11f	15g	7g	2h	15h	LL
KK				6a	15a	8b	4b	16c	15c	9d	7d	1e	14e	10f	16f	6g	18g	13h	4h	KK
JJ	4n	1c	5h	5a	14a	7b	10b	7c	2c	15d	2d	3e	13e	18f	7f	3g	2g	1h	3h	JJ
HH	1o	1z	1v	4a	13a	15b	3b	3c	18c	16d	18d	11e	6e	1f	6f	8g	5g	12h	6h	HH
GG	4y	4e	1j	3a	12a	12b	14b	9c	1c	14d	6d	10e	17e	8f	14f	11g	17g	7h	11h	GG
FF	2g	4c	4o	2a	11a	2b	16b	11c	4c	11d	8d	9e	4e	12f	15f	10g	13g	18h	14h	FF
EE	1x	3t	3u	1a	10a	9b	17b	13c	17c	17d	5d	18e	5e	5f	9f	1g	14g	5h	17h	EE
DD	4p	4r	1y	31a	2b	23c	4d	16e	28f	g-m are inoculated in September						17i	13i	5j	7j	DD
CC	4g	2p	2c	30a	11b	6c	8d	6e	7f	6g	2h	30i	18j	7k	5m	9i	18i	12j	18j	CC
BB	3j	1q	2n	29a	16b	24c	14d	5e	24f	16g	16h	10i	21j	28k	21m	6i	11i	14j	3j	BB
AA	3s	5k	3o	28a	8b	19c	31d	11e	3f	24g	6h	5i	6j	27k	8m	2i	5i	10j	1j	AA
Z	4q	1m	4v	27a	23b	18c	11d	9e	16f	27g	19h	3i	12j	14k	23m	14i	1i	8j	9j	Z
Y	1u	4i	3v	26a	9b	2c	9d	28e	6f	29g	29h	2i	28j	23k	28m	12i	8i	6j	4j	Y
X	4a	4x	1k	25a	3b	15c	7d	31e	18f	25g	27h	21i	30j	24k	13m	3i	4i	15j	11j	X
W	1f	4j	3b	24a	27b	28c	18d	24e	14f	12g	1h	24i	5j	1k	7m	15i	7i	2j	17j	W
V	2m	1s	1a	23a	18b	10c	29d	4e	10f	1g	10h	25i	2j	18k	1m	10i	12k	10m	13j	V
U	5g	1d	3p	22a	1b	4c	30d	8e	9f	31g	23h	19i	10j	21k	17m	1k	9k	8m	12m	U
T	1w	3y	1p	20a	20b	17c	15d	14e	23f	30g	3h	13i	4j	8k	31m	14k	4k	9m	6m	T
S	1n	3m	4s	19a	14b	29c	6d	27e	25f	20g	4h	23i	19j	17k	14m	10k	7k	2m	7m	S
R	4b	3f	3e	18a	17b	8c	26d	29e	15f	28g	8h	20i	17j	29k	30m	13k	15k	15m	11m	R
Q	3h	1i	5j	17a	10b	30c	27d	22e	30f	17g	12h	8i	7j	10k	27m	11k	18k	13m	18m	Q
P	2a	3x	5b	16a	19b	12c	16d	30e	19f	3g	22h	22i	8j	4k	18m	17k	6k	4m	14m	P
O	4u	5e	2j	15a	15b	22c	3d	25e	20f	7g	7h	6i	27j	22k	24m	8k	2k	1m	5m	O
N	3a	5c	3i	14a	30b	26c	19d	15e	31f	21g	20h	7i	22j	5k	29m	5k	3k	3m	17m	N
M	4w	3z	4t	12a	7b	31c	12d	7e	5f	13g	13h	1i	23j	2k	16m	11a	3b	2c	4d	M
L	3n	1l	3g	11a	25b	9c	22d	23e	11f	8g	24h	31i	13j	12k	20m	10a	9b	1c	11d	L
K	3l	2e	4h	10a	5b	7c	23d	2e	12f	4g	14h	14i	1j	6k	22m	9a	6b	6c	10d	K
J	1e	1r	3r	9a	6b	5c	5d	19e	1f	18g	15h	28i	14j	13k	25m	8a	11b	3c	5d	J
H	2b	4l	5a	8a	28b	14c	1d	26e	27f	23g	21h	18i	24j	20k	19m	7a	4b	11c	3d	H
G	4d	5f	5d	7a	29b	27c	24d	1e	22f	10g	17h	12i	20j	16k	10m	6a	2b	5c	2d	G
F	1g	4z	2o	6a	22b	1c	10d	17e	17f	15g	18h	4i	29j	31k	2m	5a	7b	4c	9d	F
E	3k	5i	4f	5a	4b	3c	25d	12e	4f	5g	5h	27i	16j	25k	15m	4a	10b	10c	6d	E
D	1t	3w	4k	4a	31b	16c	2d	3e	26f	22g	30h	16i	15j	15k	6m	3a	1b	8c	7d	D
C	1b	2l	5l	3a	12b	11c	17d	20e	2f	14g	25h	29i	31j	3k	4m	2a	5b	9c	8d	C
B	3d	4m	2r	2a	26b	25c	28d	10e	29f	19g	28h	17i	25j	19k	3m	1a	8b	7c	1d	B
A	3q	2i	2k	1a	24b	20c	20d	18e	8f	2g	31h	15i	3j	30k	12m					A
	2d	3c	2f																	
	1h	2q	2h																	
Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Cell
	Rows																			

Cultivar screening
Field experiments 2008 and 2009

Cultivars	Genome[b]	2008	2009	2008-2009 mean
Ettinger	GxM	n/t	2.9	2.9
Hass	GxM	3.9	2.9	3.4
Winter Mexican	GxM	n/t	1.8	1.8
Bacon	G	n/t	2.2	2.2
Reed	G	n/t	3.5	3.3
Brogdon	GxMxWI	4.1	4.1	4.1
Beta	GxWI	n/t	3.2	3.2
Choquette	GxWI	3.5	3.6	3.6
Hall	GxWI	3.3	4.9	4.1
Lula	GxWI	5.5	3.1	4.3
Miguel	GxWI	6.4	3.7	5.1
Monroe	GxWI	5.2	2.9	4.1
Tonnage	GxWI	n/t	3.5	3.5
Bernecker	WI	5.2	4.2	4.7
Catalina	WI	5.1	5.1	5.1
Day	WI	4.4	n/t	4.4
Donnie	WI	6.3	4.5	5.4
Pollack	WI	n/t	3.7	3.7
Russell	WI	n/t	5.6	5.6
Simmonds	WI	6.3	5.8	6.1
Trapp	WI	n/t	3.3	3.3
Waldin	WI	n/t	4.3	4.3

12-18 expt units/yr; severity rated on 1-10 subjective scale

Cultivar screening
Field experiments 2008 and 2009

Cultivars	Genome[b]	2008	2009	2008-2009 mean
Ettinger	GxM	n/t	2.9	2.9
Hass	GxM	3.9	2.9	3.4
Winter Mexican	GxM	n/t	1.8	1.8
Bacon	G	n/t	2.2	2.2
Reed	**G**	**n/t**	**3.5**	**3.3**
Brogdon	**GxMxWI**	**4.1**	**4.1**	**4.1**
Beta	**GxWI**	**n/t**	**3.2**	**3.2**
Choquette	**GxWI**	**3.5**	**3.6**	**3.6**
Hall	**GxWI**	**3.3**	**4.9**	**4.1**
Lula	**GxWI**	**5.5**	**3.1**	**4.3**
Miguel	**GxWI**	**6.4**	**3.7**	**5.1**
Monroe	**GxWI**	**5.2**	**2.9**	**4.1**
Tonnage	**GxWI**	**n/t**	**3.5**	**3.5**
Bernecker	WI			
Catalina	WI	5.1	5.1	5.1
Day	WI	4.4	n/t	4.4
Donnie	**WI**	**6.3**	**4.5**	**5.4**
Pollack	**WI**	**n/t**	**3.7**	**3.7**
Russell	**WI**	**n/t**	**5.6**	**5.6**
Simmonds	**WI**	**6.3**	**5.8**	**6.1**
Trapp	WI	n/t	3.3	3.3
Waldin	**WI**	**n/t**	**4.3**	**4.3**

Boldface cultivar = recommended for Florida

Cultivar screening
Field experiments 2008 and 2009

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Ettinger	GxM	n/t	2.9	2.9	
Hass	GxM	3.9	2.9	3.4	
Winter Mexican	GxM	n/t	1.8	1.8	**2.7 b**
Bacon	G	n/t	2.2	2.2	
Reed	G	n/t	3.5	3.3	**2.8 b**
Brogdon	GxMxWI	4.1	4.1	4.1	**4.1 ab**
Beta	GxWI	n/t	3.2	3.2	
Choquette	GxWI	3.5	3.6	3.6	
Hall	GxWI	3.3	4.9	4.1	
Lula	GxWI	5.5	3.1	4.3	
Miguel	GxWI	6.4	3.7	5.1	
Monroe	GxWI	5.2	2.9	4.1	
Tonnage	GxWI	n/t	3.5	3.5	**4.0 ab**
Bernecker	WI	5.2	4.2	4.7	
Catalina	WI	5.1	5.1	5.1	
Day	WI	4.4	n/t	4.4	
Donnie	WI	6.3	4.5	5.4	
Pollack	WI	n/t	3.7	3.7	
Russell	WI	n/t	5.6	5.6	
Simmonds	WI	6.3	5.8	6.1	
Trapp	WI	n/t	3.3	3.3	
Waldin	WI	n/t	4.3	4.3	**4.7 a**

WI cultivars most susceptible

Cultivar screening
Field experiments 2008 and 2009

Cultivars	Genome[b]	2008	2009	2008-2009 mean	Genome mean[d]
Ettinger	GxM	n/t	2.9	2.9	
Hass	GxM	3.9	2.9	3.4	
Winter Mexican	GxM	n/t	1.8	1.8	**2.7 b**
Bacon	G	n/t	2.2	2.2	
Reed	G	n/t	3.5	3.3	**2.8 b**
Brogdon	GxMxWI	4.1	4.1	4.1	**4.1 ab**
Beta	GxWI	n/t	3.2	3.2	
Choquette	GxWI	3.5	3.6	3.6	
Hall	GxWI	3.3	4.9	4.1	
Lula	GxWI	5.5	3.1	4.3	
Miguel	GxWI	6.4	3.7	5.1	
Monroe	GxWI	5.2	2.9	4.1	
Tonnage	GxWI	n/t	3.5	3.5	**4.0 ab**
Bernecker	WI	5.2	4.2	4.7	
Catalina	WI	5.1	5.1	5.1	
Day	WI	4.4	n/t	4.4	
Donnie	WI	6.3	4.5	5.4	
Pollack	WI	n/t	3.7	3.7	
Russell	WI	n/t	5.6	5.6	
Simmonds	WI	6.3	5.8	6.1	
Trapp	WI	n/t	3.3	3.3	
Waldin	WI	n/t	4.3	4.3	**4.7 a**

WI cultivars most susceptible

Impact of G and M backgrounds?

Cultivar screening
Field experiments 2008 and 2009

Cultivars	Genome[b]	2008	2009	2008-2009 mean	Genome mean[d]
Ettinger	GxM	n/t	2.9	2.9	
Hass	GxM	3.9	2.9	3.4	
Winter Mexican	GxM	n/t	1.8	1.8	**2.7 b**
Bacon	G	n/t	2.2	2.2	
Reed	G	n/t	3.5	3.3	**2.8 b**
Brogdon	GxMxWI	4.1	4.1	4.1	**4.1 ab**
Beta	GxWI	n/t	3.2	3.2	
Choquette	GxWI	3.5	3.6	3.6	
Hall	GxWI	3.3	4.9	4.1	
Lula	GxWI	5.5	3.1	4.3	
Miguel	GxWI	6.4	3.7	5.1	
Monroe	GxWI	5.2	2.9	4.1	
Tonnage	GxWI	n/t	3.5	3.5	**4.0 ab**
Bernecker	WI	5.2	4.2	4.7	
Catalina	WI	5.1	5.1	5.1	
Day	WI	4.4	n/t	4.4	
Donnie	WI	6.3	4.5	5.4	
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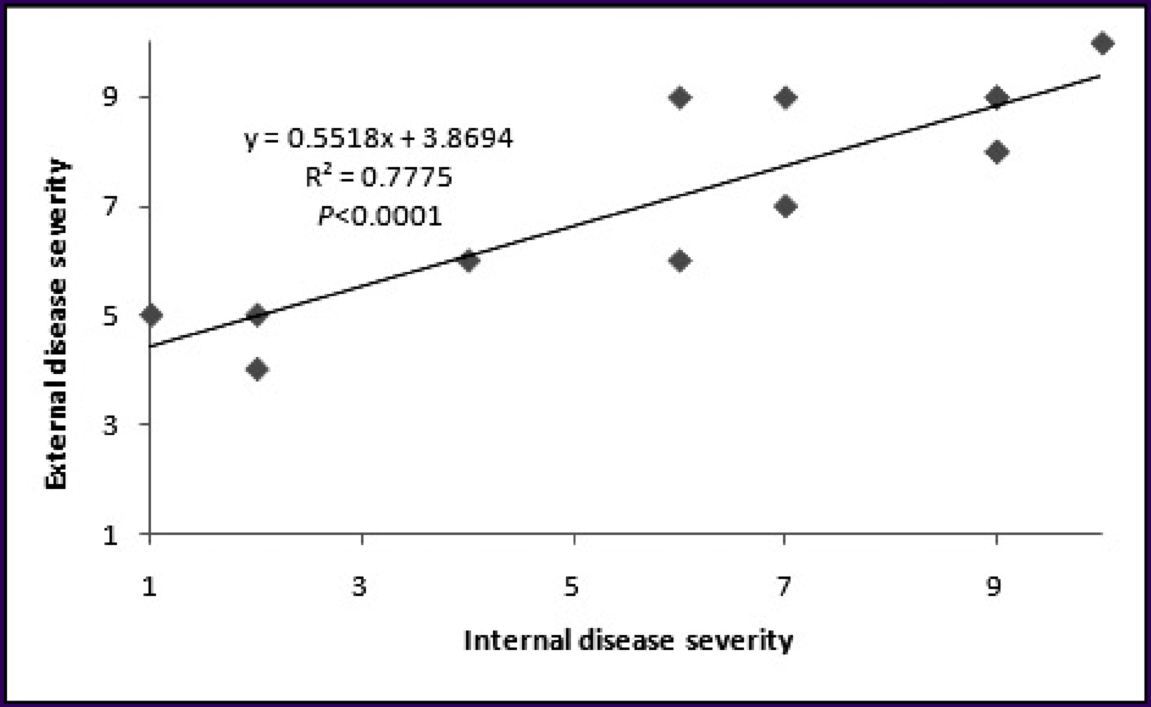
Tolerance may require a G and/or M pedigree

Host:pathogen interactions?

- Internal and external disease development correlated



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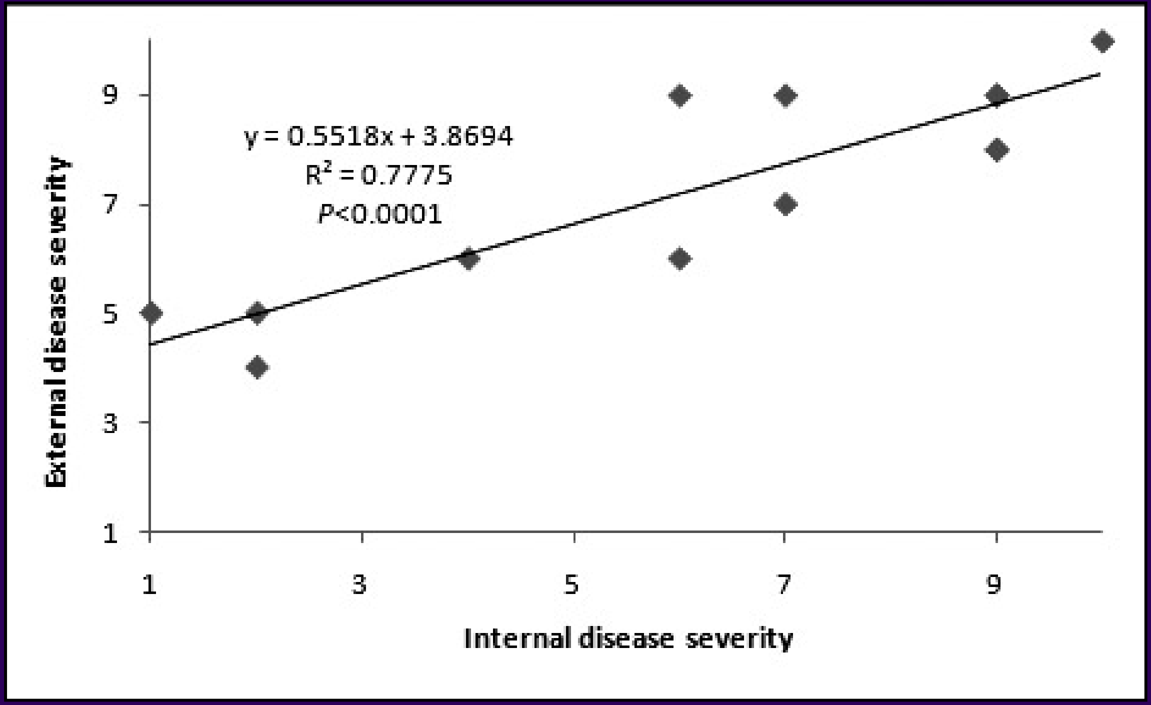

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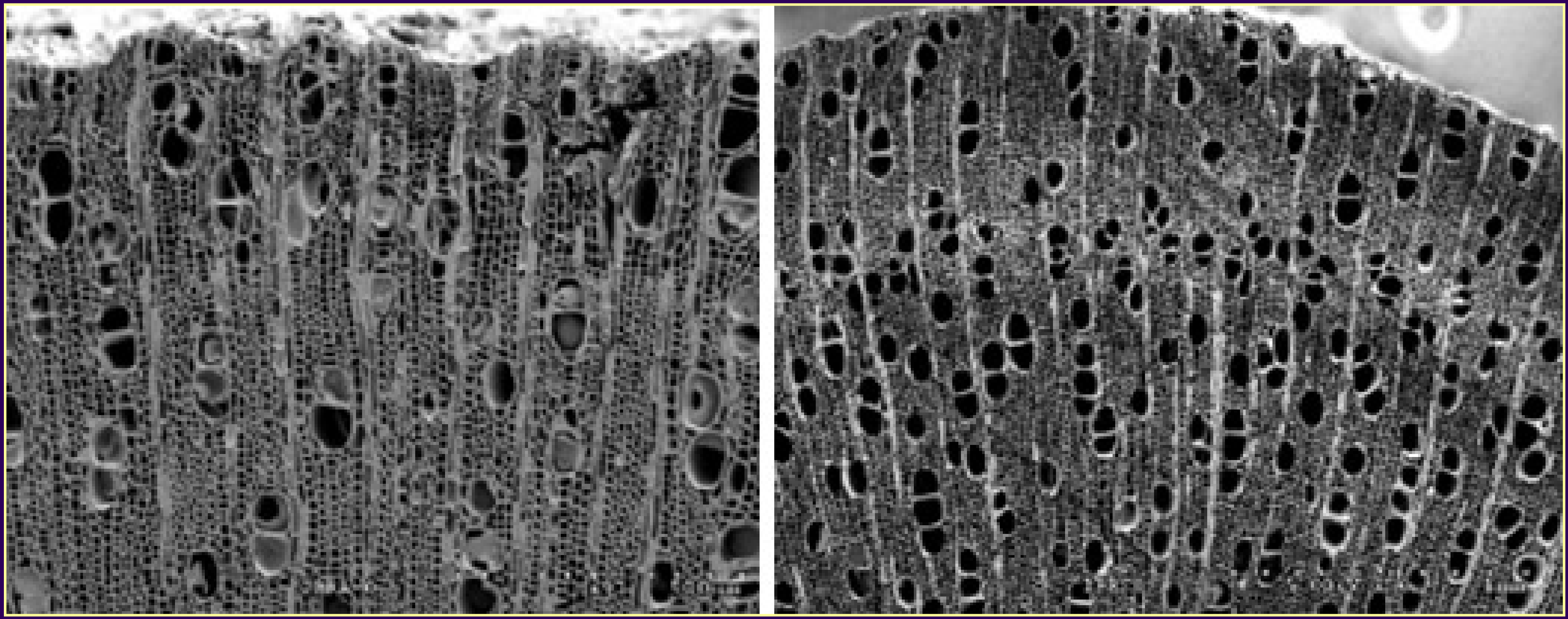
- Macroscropic and microscopic reactions of susceptible and tolerant cvs of avocado and other host species against this disease?



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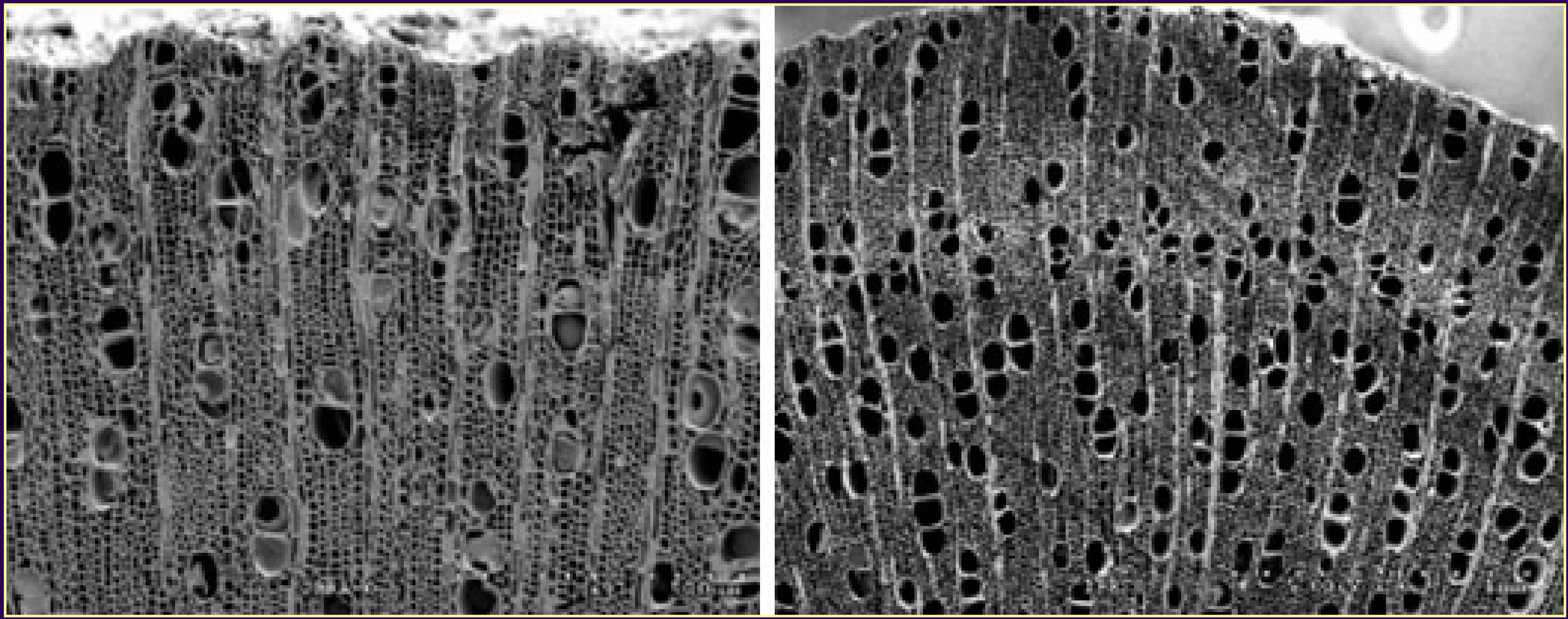

•Avocado responds to *R. lauricola* by accumulating phenolic substances and producing tyloses (typical host defense responses); ultimately, indicators of resistance are sought in this work

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28 different fungicides in 18 chemical groups tested for *in vitro* activity against *R. lauricola*

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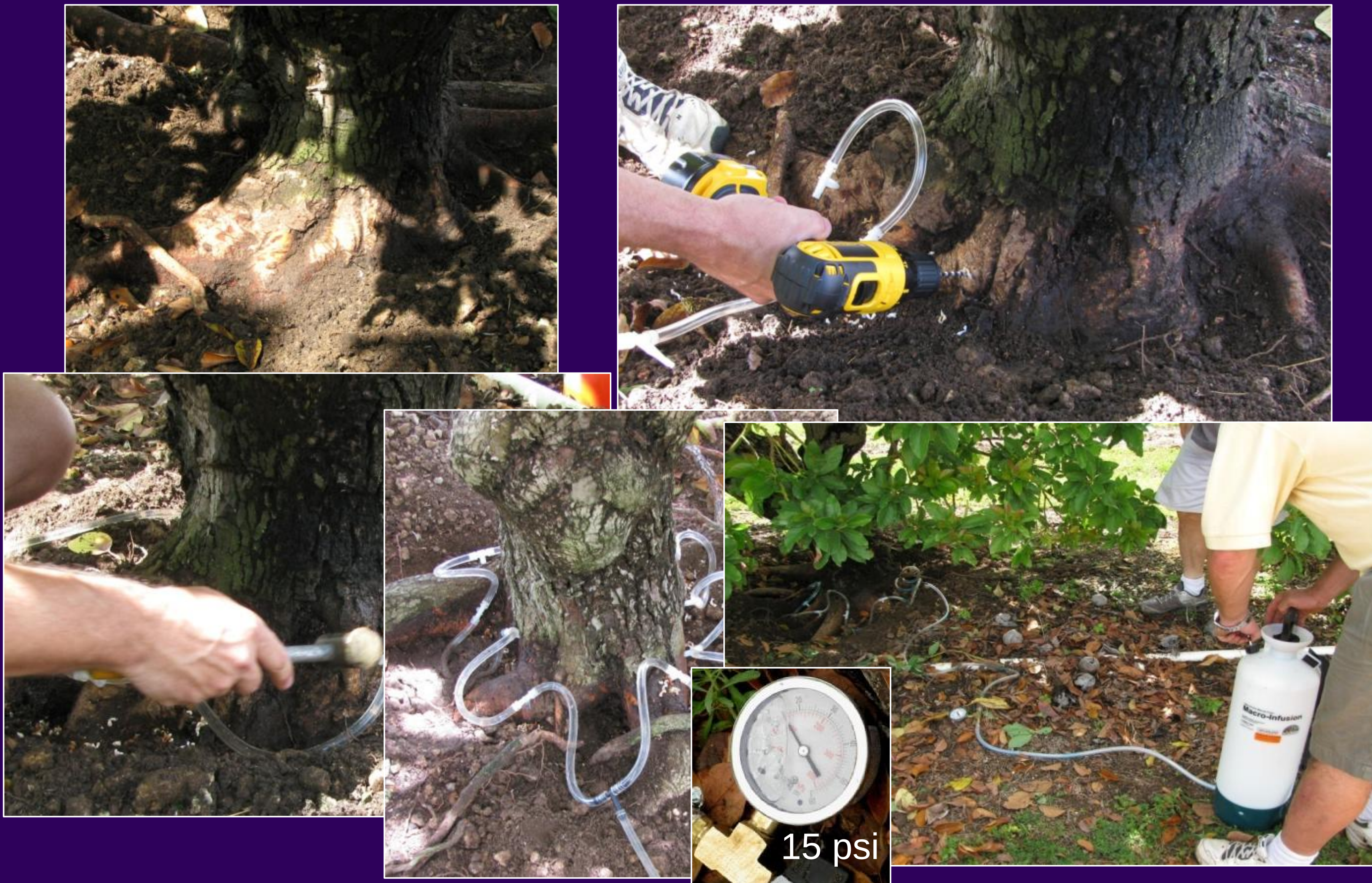
Topical (trunk and branch) applications of propiconazole+surfactants are being studied for xylem loading (macro-infusion is efficient, but too expensive)

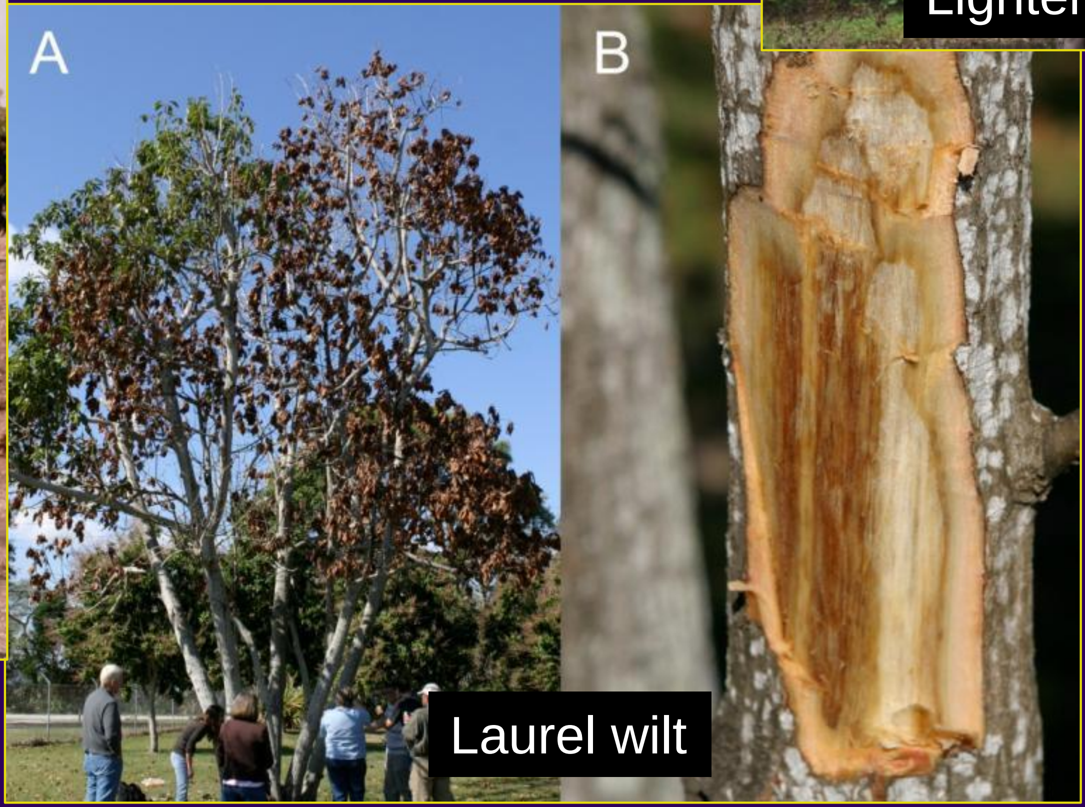
Macro-infusion of fungicides



Laurel wilt diagnosis

Symptoms

- Not very accurate

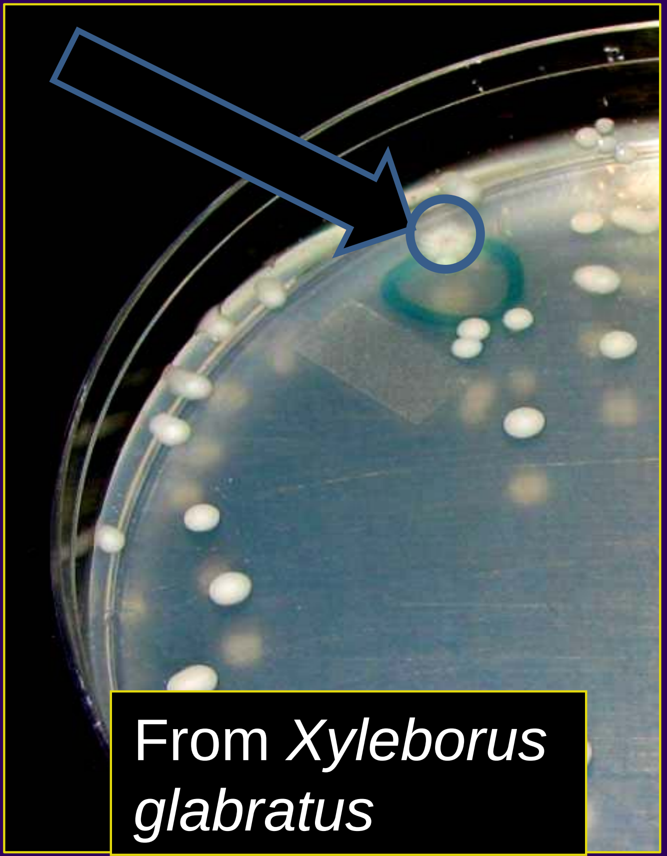


Laurel wilt diagnosis

Isolation of *Raffaelea lauricol*a on Ophiostoma semi-selective medium

- More accurate, but not specific

From diseased plants



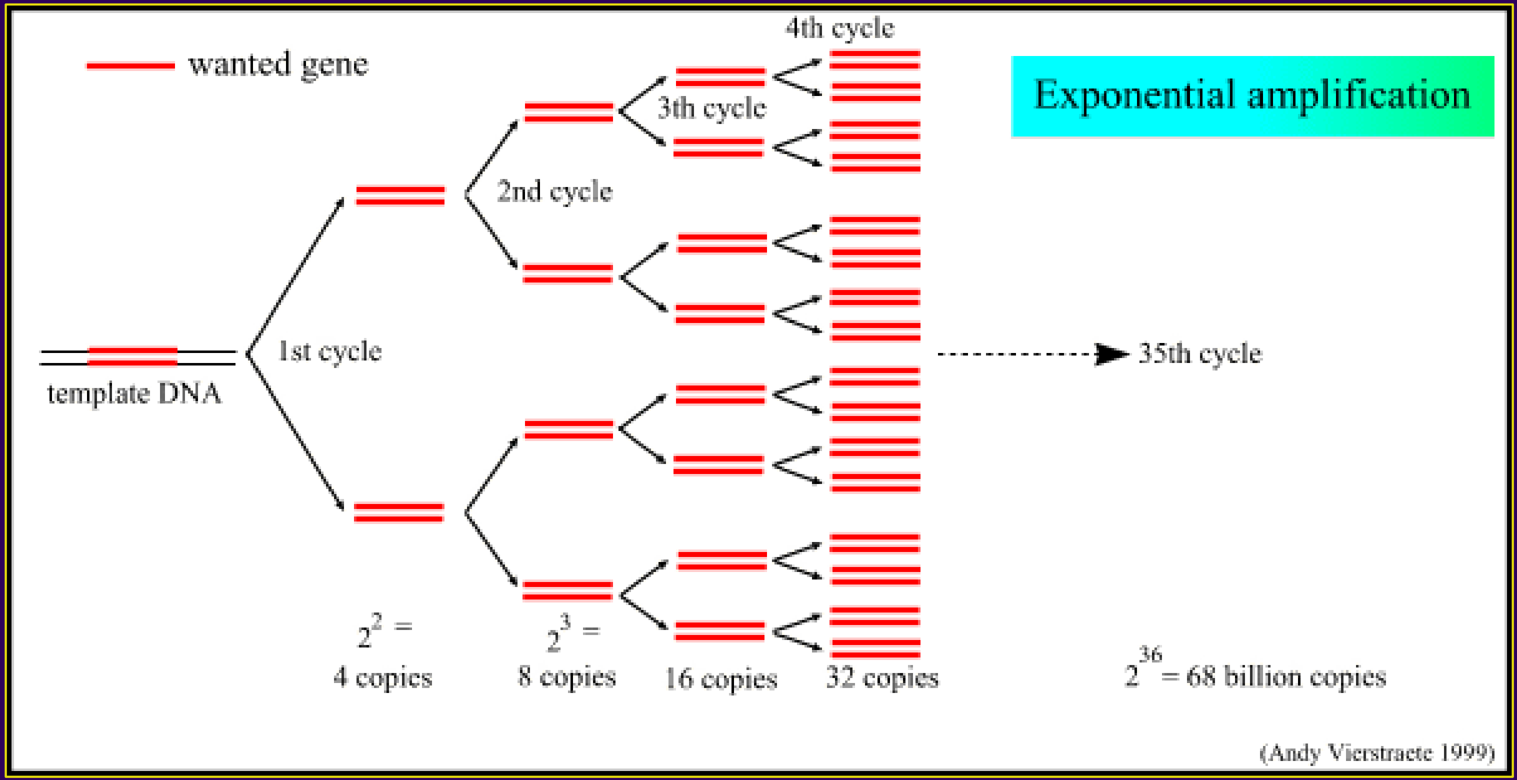
From *Xyleborus glabratus*

Laurel wilt diagnosis

DNA-based diagnoses

•Can be very accurate

Polymerase Chain Reaction



http://users.ugent.be/~avierstr/principles/pcr.html

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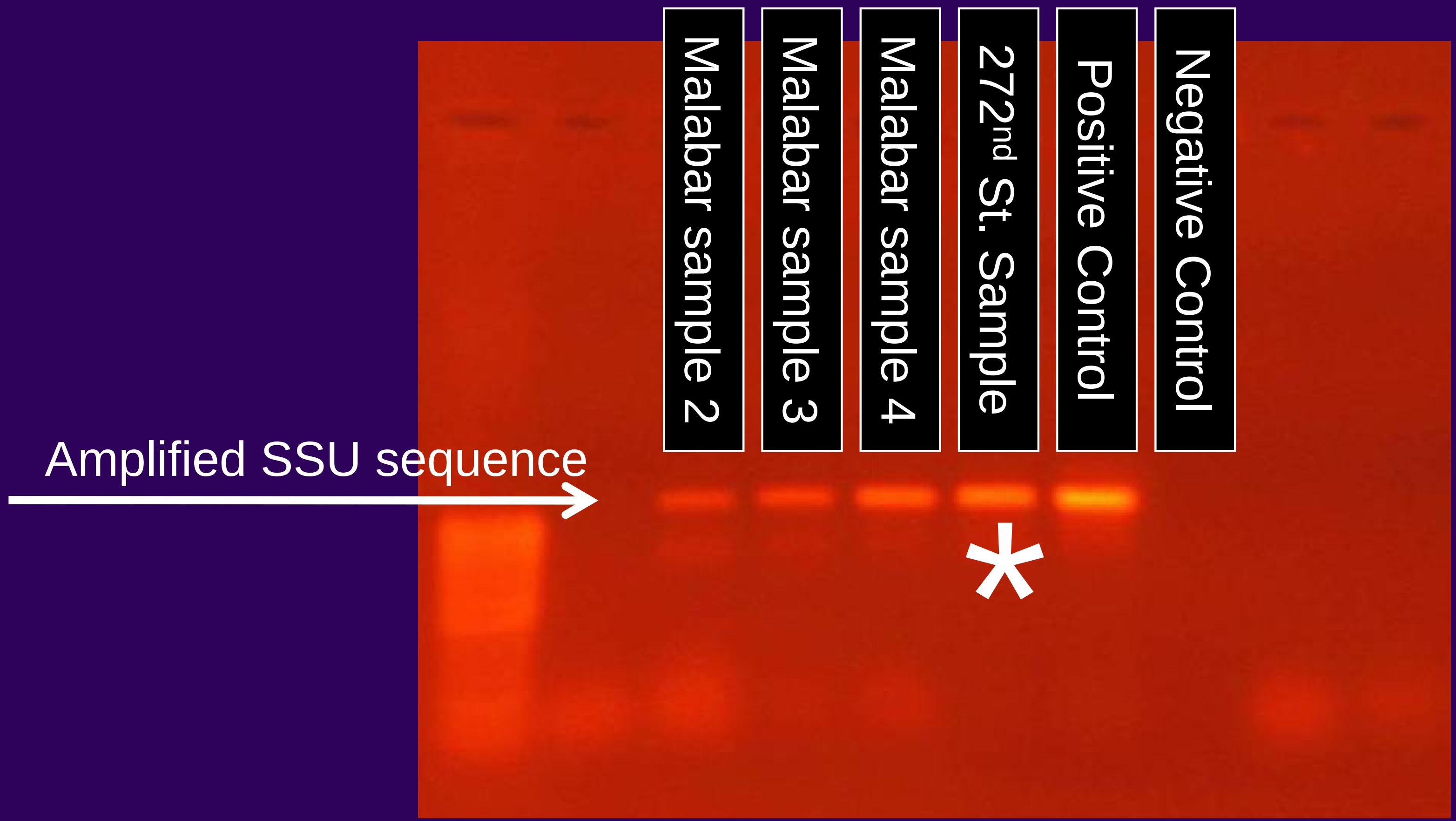
Traditional PCR Results

(diagnostic SSU primers are generally selective)



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- A highly specific diagnostic tool, now under development, will play important role in interdiction and laurel wilt management via sanitation
- Early detection of the pathogen and disease are needed quarantine, eradication and sanitation efforts

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- Germplasm from affected areas should <u>not</u> be imported from affected areas unless its pathogen- and vector-free status can be confirmed
- Diagnostic tools used to study the disease and pathogen could also be used in disease-free certification efforts
- Laurel wilt will be a difficult management problem: fungicides, tolerant germplasm, sanitation and various chemicals for managing the insect vector may ultimately all be useful

Special thanks to:

- CFCS meeting organizers
- USDA, T-STAR

Additional thanks to:

- Florida Avocado Committee
- University of Florida, IFAS Vice President
- Pine Island Nursery
- Miami-Dade County Commission
- Zill High Performance Plants, Inc.
- USDA , APHIS-PPQ
- USDA, CSREES/NIFA, SCRI