



Strengthening Avocado Disease Surveillance through Enhanced Diagnostics for Scab and Laurel Wilt

Romina Gazis, Ph.D.

Associate Professor & Extension Specialist

Director, Plant Diagnostic Clinic

Department of Plant Pathology, University of Florida

Tropical Research and Education Center



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We will focus on two diseases due to their importance in biosecurity and the severity of damage they can cause

1



SCAB

2



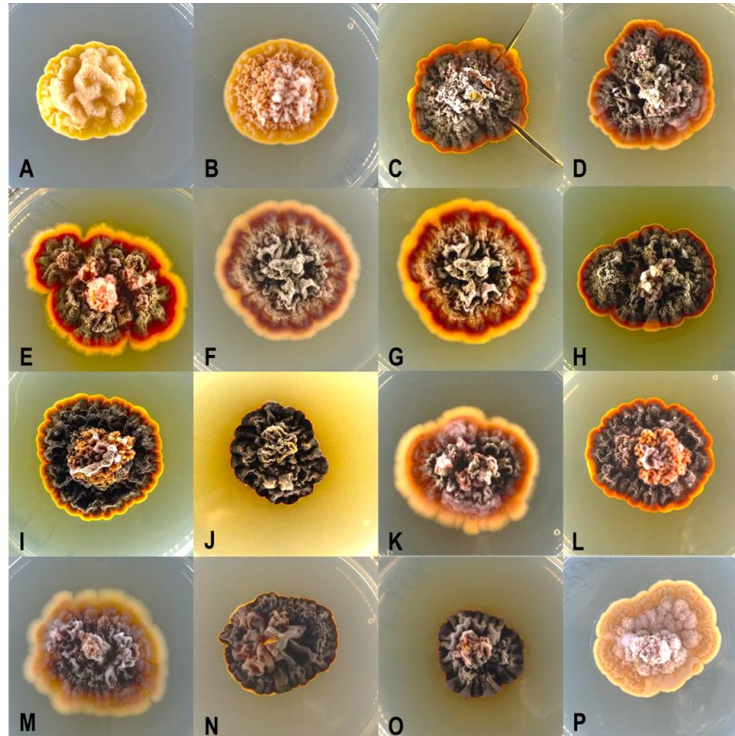
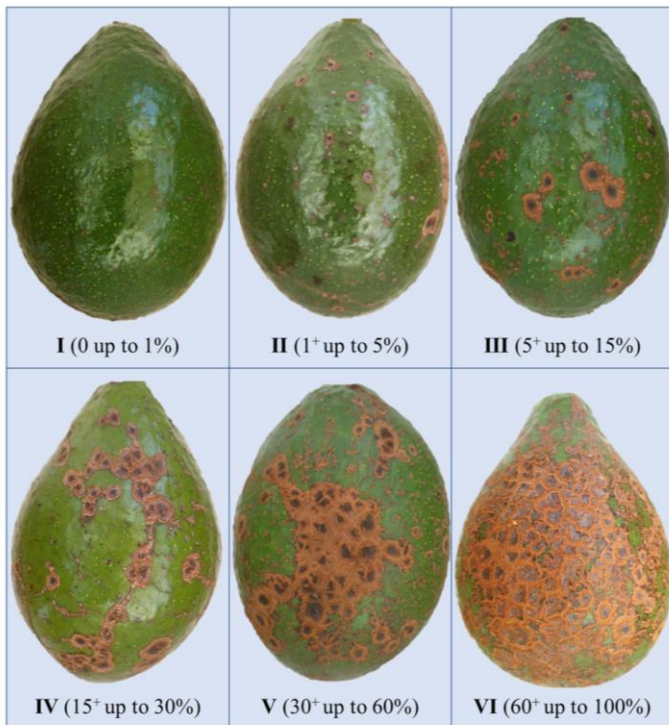
LAUREL WILT



Disease Diagnostic Guide

Avocado Scab:

Elsinoe perseae (anamorph *Sphaceloma perseae*)



Fruit symptoms include superficial, corky, raised lesions that are brown to purple, oval or irregular in shape; over time, they dry and crack, allowing secondary fungi to enter and cause fruit rot



cv. Lima Late



cv. Lula



cv. Hass



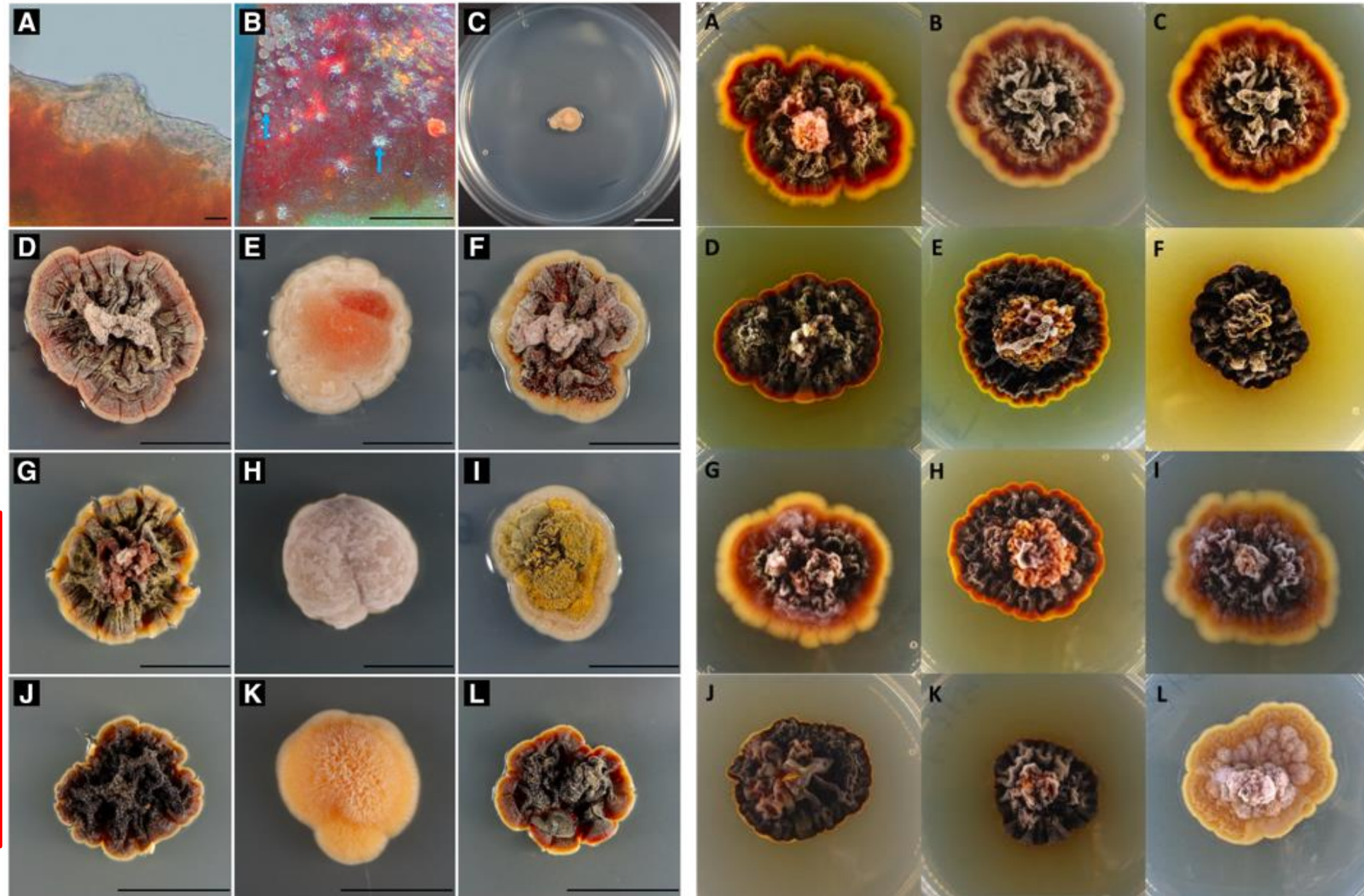
Symptoms are nonspecific, as they can also result from mechanical damage, and they vary by avocado cultivar



Fruits with mechanical/thrip damage, Hass Orchard, Mexico

FIGURE 2

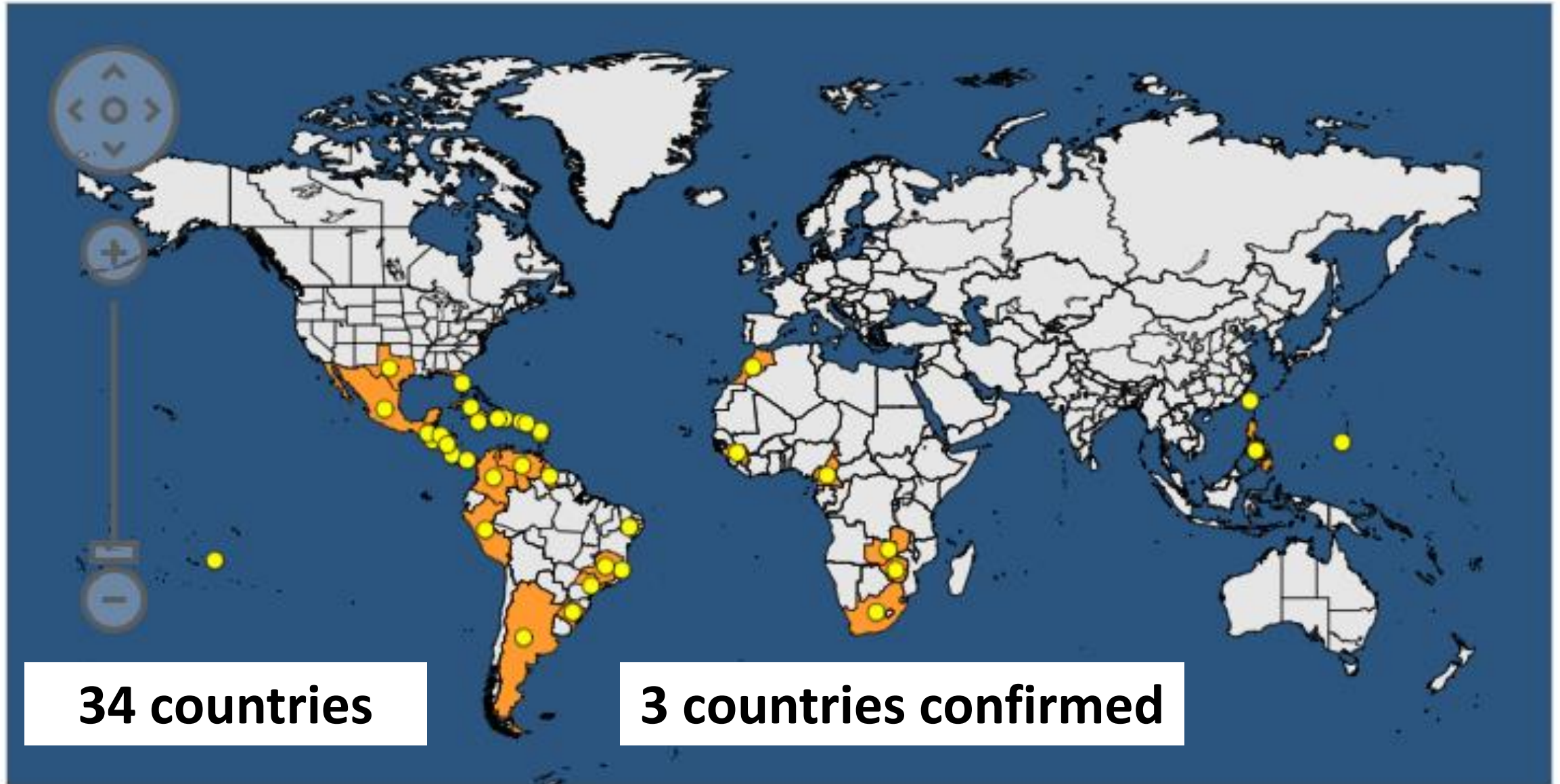
Microscopic and macroscopic observations of *Elsinoe perseae*. **A**, Acervulus and (blue arrows in **B**) small colonies of *E. perseae* erupting from host infected tissue. Morphology of *E. perseae* cultures after **C**, 1 or **D to L**, 3 weeks grown on different culture media and under different photoperiod conditions as follows: **D to F**, potato dextrose agar (PDA) media under a 12-h near-UV-light photoperiod, **G to I**, PDA media under complete darkness, and **J to L**, malt extract agar media under complete darkness. Isolates shown include **D, G, and J**, TREC-ASLu3, **E, H, and K**, TREC-ILL8, and **F, I, and L**, TREC-ASLu6. Scale bars: **A** = 10 μ m; **B** = 1 mm; **C to L** = 10 mm.



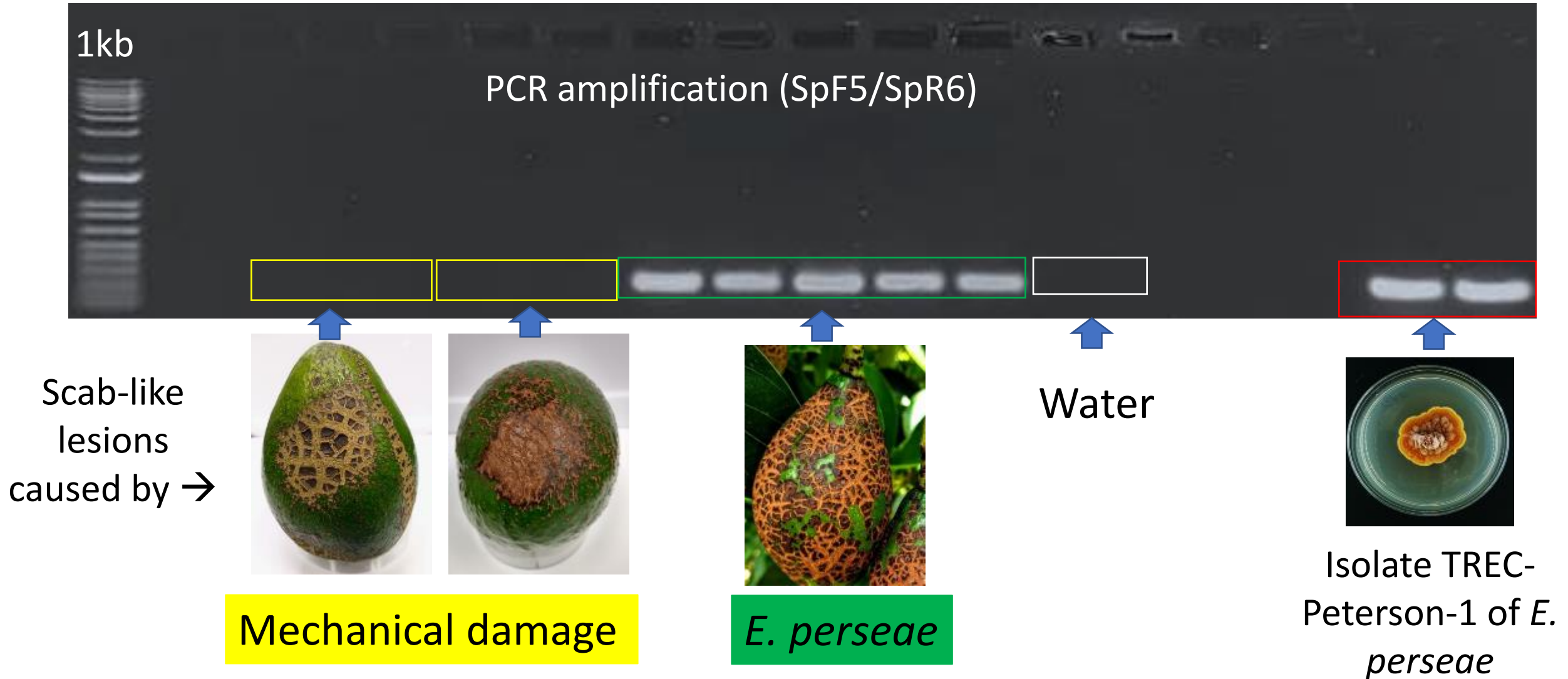
**Difficult to
isolate, slow-
growing, and
morphologically
plastic**

All *Elsinoe perseae* isolates, 4 weeks-old, on PDA

Distribution



Rapid detection of *Elsinoe perseae* DNA directly from symptomatic fruit peel



Development of a qPCR assay for the rapid detection of the avocado scab fungus
Elsinoe perseae
 --Manuscript Draft--

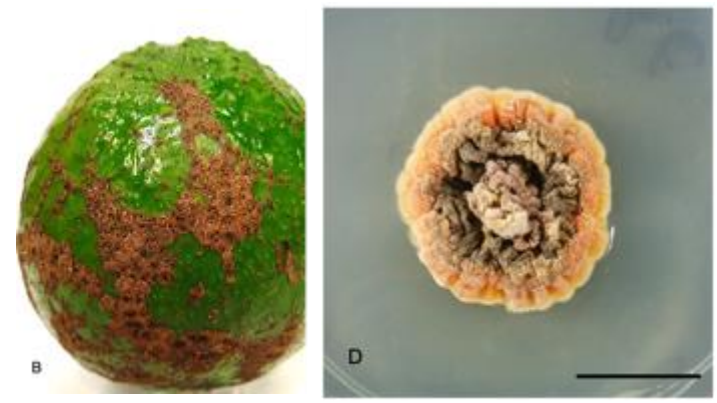
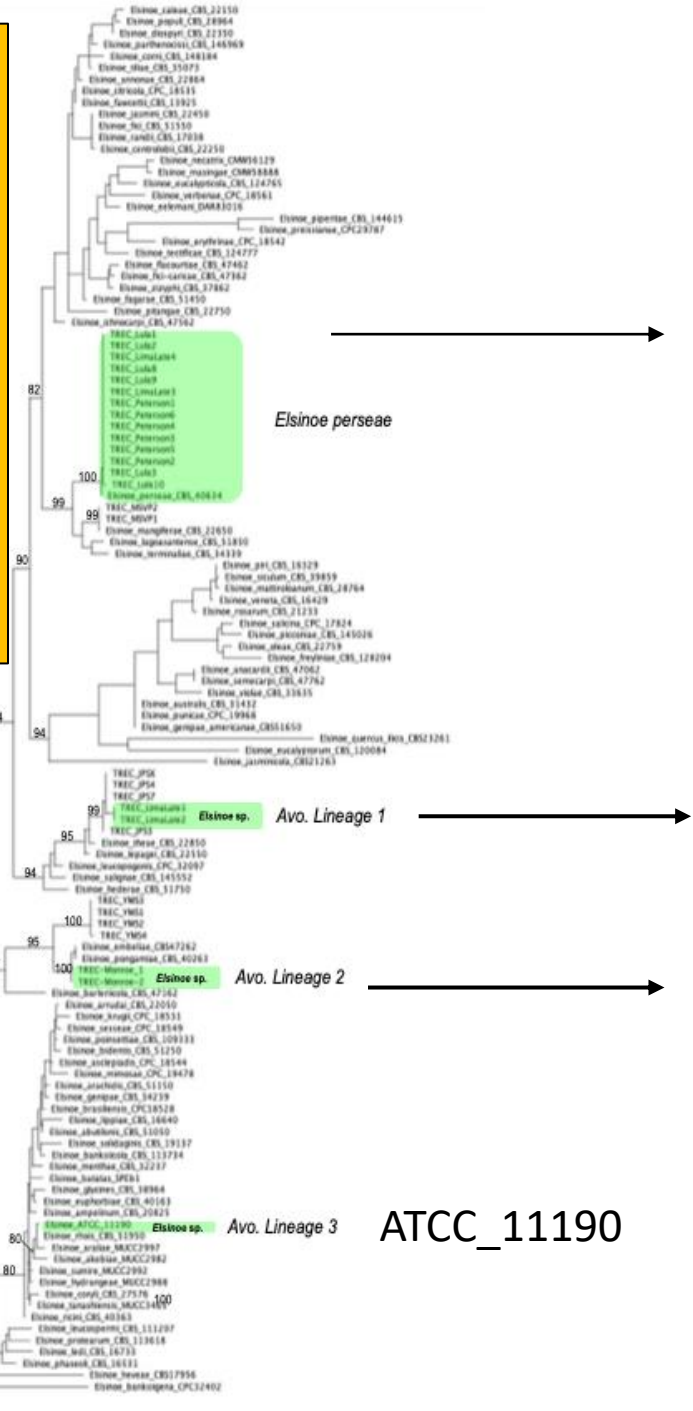
Manuscript Number:	
Full Title:	Development of a qPCR assay for the rapid detection of the avocado scab fungus <i>Elsinoe perseae</i>
Article Type:	Original Research Article
Keywords:	Quantitative real time PCR, <i>Persea americana</i> , ITS, RPB2, internal transcribed space (ITS), DNA-directed RNA polymerase subunit II (RPB2)
Corresponding Author:	Nga Thi Tran, PhD University of Queensland Dutton Park, Queensland AUSTRALIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Queensland
Corresponding Author's Secondary Institution:	
First Author:	Nga Thi Tran, PhD
First Author Secondary Information:	
Order of Authors:	Nga Thi Tran, PhD Louisamarie E. Parkinson Lara-Simone Pretorius Romina Gazis Lederson Gañán-Betancur Samuel Garty Andrew D. W. Geering

qPCR Duplex (ITS, RPB2) assay,
under review

493 **Table 2.** TaqMan qPCR primer and probes, and synthetic DNA controls developed and used in the study.

Name	Target loci	Sequence (5' - 3')	5' Modification	3' Modification	Amplicon size (bp)
Primers and probes					
eITSCalFluor	ITS	TTGTAGTCGGAGTACAACCGT	CAL Fluor560	BHQ1	252
eITS-F		CGAACCAACTCTTGACATCA	-	-	-
eITS-R		GCCACACGCCAATACCAA	-	-	-
eRPB2FAM	RPB2	TGAATGGACAATGGATCGGTG	FAM	BHQ1	222
eRPB2-F		ACTAACCCAAATGCCACTAAG	-	-	-
eRPB2-R		GTCACCAGGCTCGTTATTGA	-	-	-
Synthetic DNA controls					
EpITS-gB		GACAGCGCCCGCCGGGGGACC GAACCAACTCTTGACATCAAA CATTGTAGTCGGAGTACAACCG TACAATCAAATCAAACCTTTCA ACAACGGATCTCTTGTTCTGG CATCGATGAAGAACGCAGCGA AATGCGATAAGTAATGTGAATT GCAGAATTCAGTGAATCATCGA ATCTTTGAACGCACATTGCGCC CCTTGGTATTCCGAGGGGCATG CCTGTTGAGCGTCATTTACC AATCAAGCCCCGCTTGGTATTG GGCGTGTGGCCGGCCCCCCCCG TGGCCGGC	-	-	291
EpRPB2-gB		GAATTCCTGGAAGAGTACGAT CCAAAGACTAACCCAAATGCCA CTAAGGTGTTTGTGAATGGACA ATGGATCGGTGTCACCAGAGA CCCCGCCAGCTTGACGAGACC ATCCTCAATCTCAGACGCCACG ATACACTTTCCTCGAAATCAG TTTGATCCGCGACGTCCGAGCA CGTGAGTCCGCATCTTACAG ATTGCGGTGCGTGATGAGAC CCGTCTTTGTCGTCAATAACGA GCCTGGTGACGACCAGGGCAA GCTTTACTTCA	-	-	271

Two lineages of unidentified *Elsinoe* species causing scab symptoms on avocado



True *Elsinoe perseae*



Lima Late & Peterson cv.



Monroe cv.

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1



SCAB

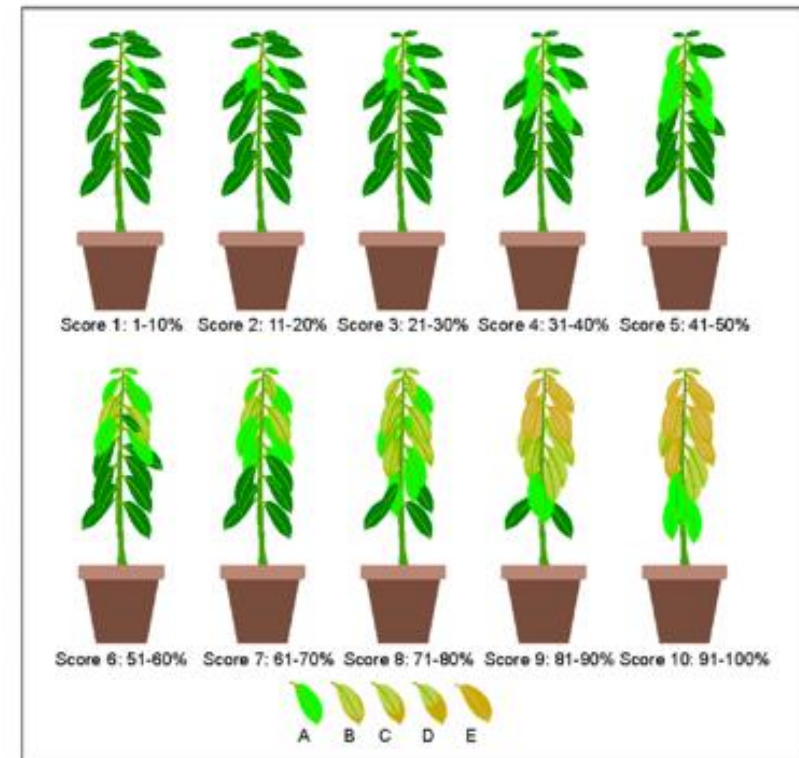
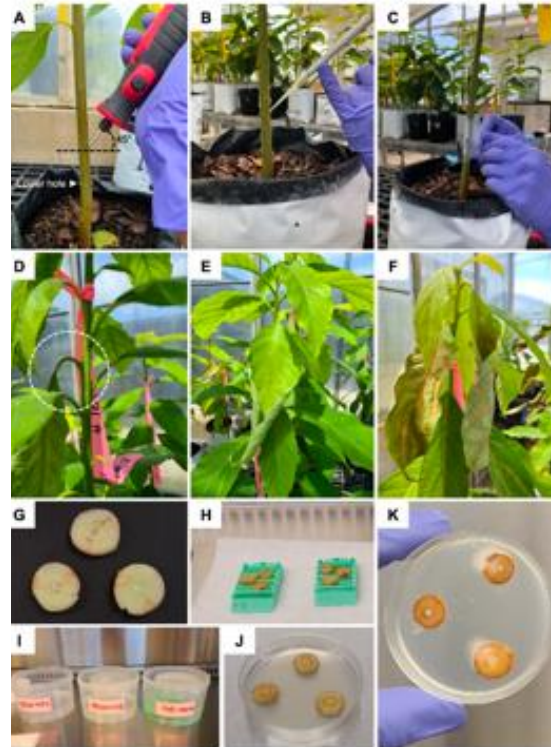
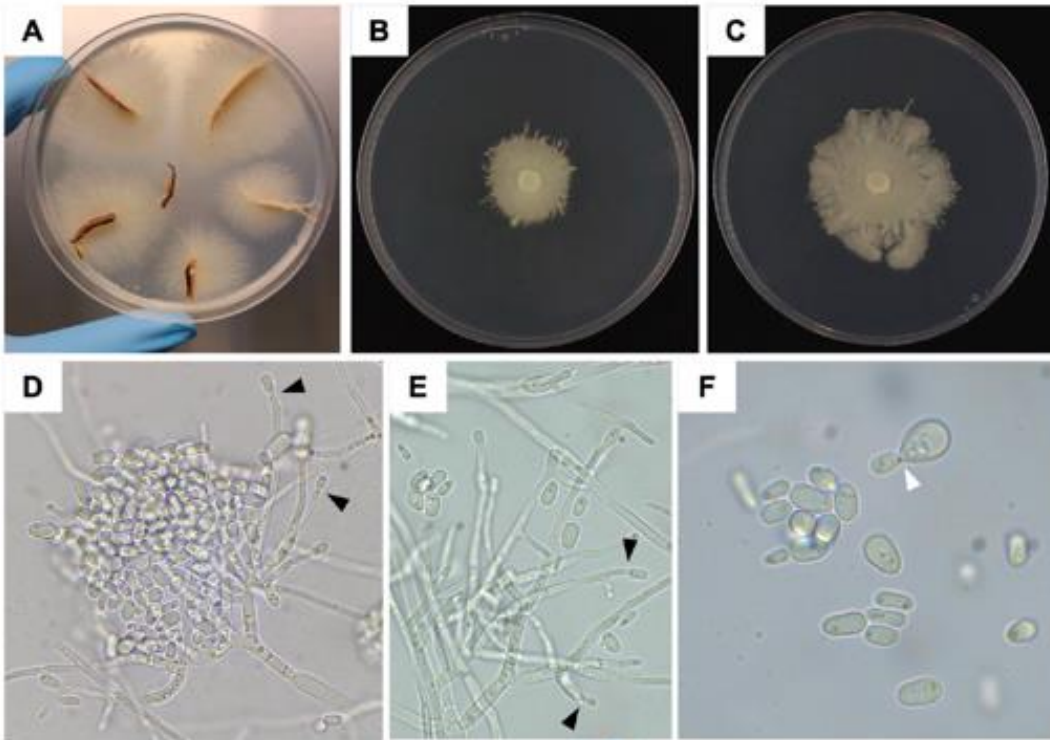
2



LAUREL WILT



Disease Diagnostic Guide: Laurel Wilt Disease in Avocado



Laurel Wilt (LW) – Disease Complex

Harringtonia lauricola
(causal agent)



Xyleborus glabratus
(Redbay Ambrosia Beetle)



0.5 mm

Average #CFUs: 6,000



"Farmer beetles"



Average #CFUs: <100

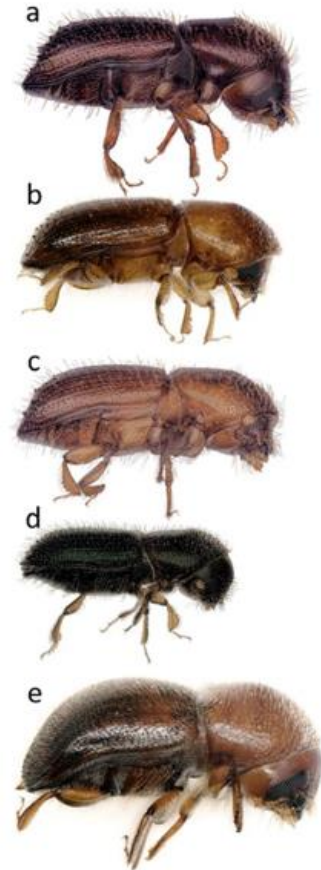
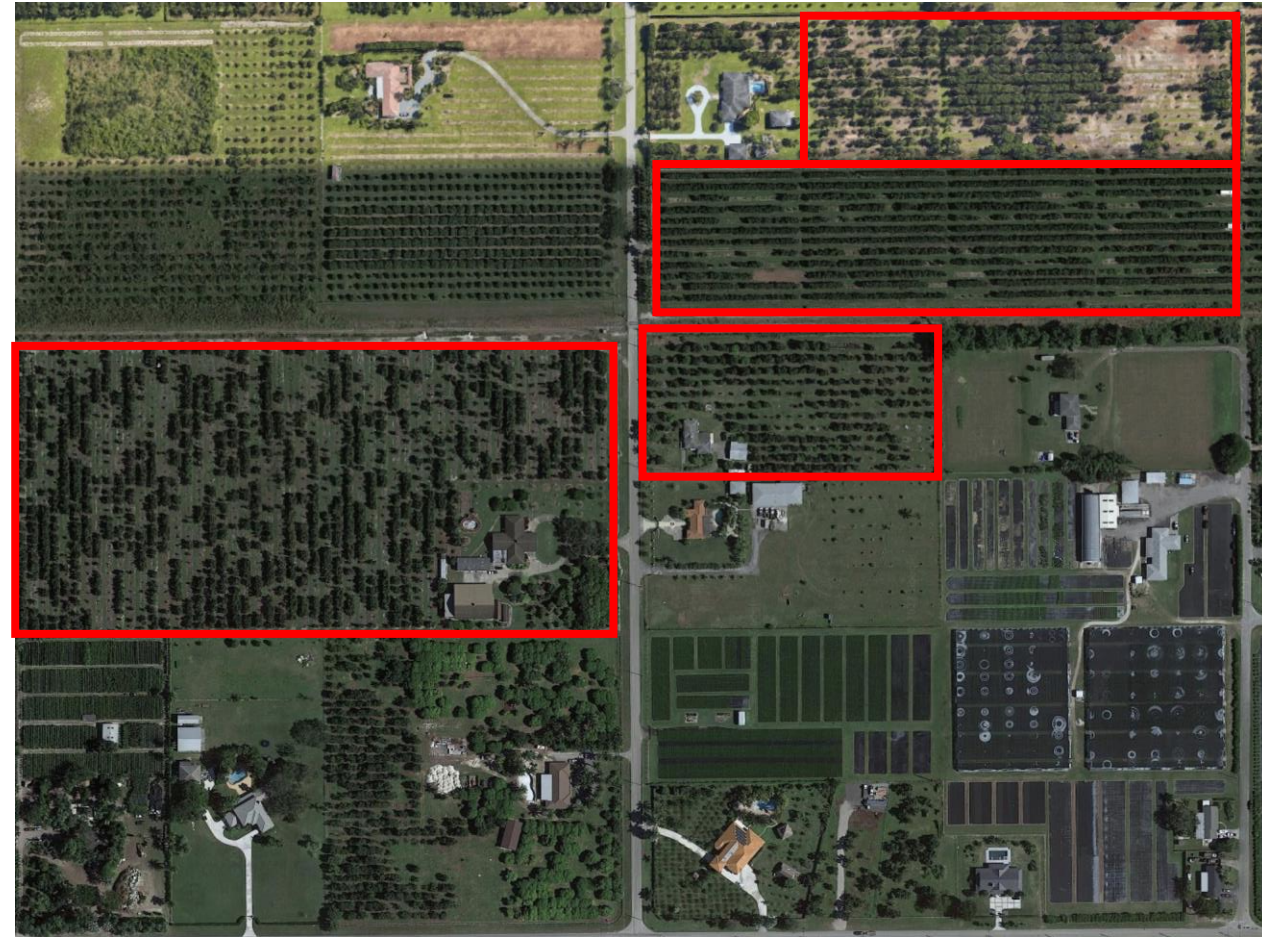


Fig. 1 Lateral view of five ambrosia beetle species associated with *R. lauricola* in avocado. **a** *Xyleborus bispinatus*, **b** *Xyleborus affinis*, **c** *Xyleborus volvulus*, **d** *Xyleborinus saxesenii*, **e** *Xylosandrus crassiusculus*. Note that the size of the beetles is an approximation. The body length of these beetle species can be found in Gomez et al. 2018

Figure from Cruz et al. 2021. Symbiosis
<https://doi.org/10.1007/s13199-021-00776-2>

LAUREL WILT HAS DEVASTATED THE AVOCADO INDUSTRY IN FLORIDA

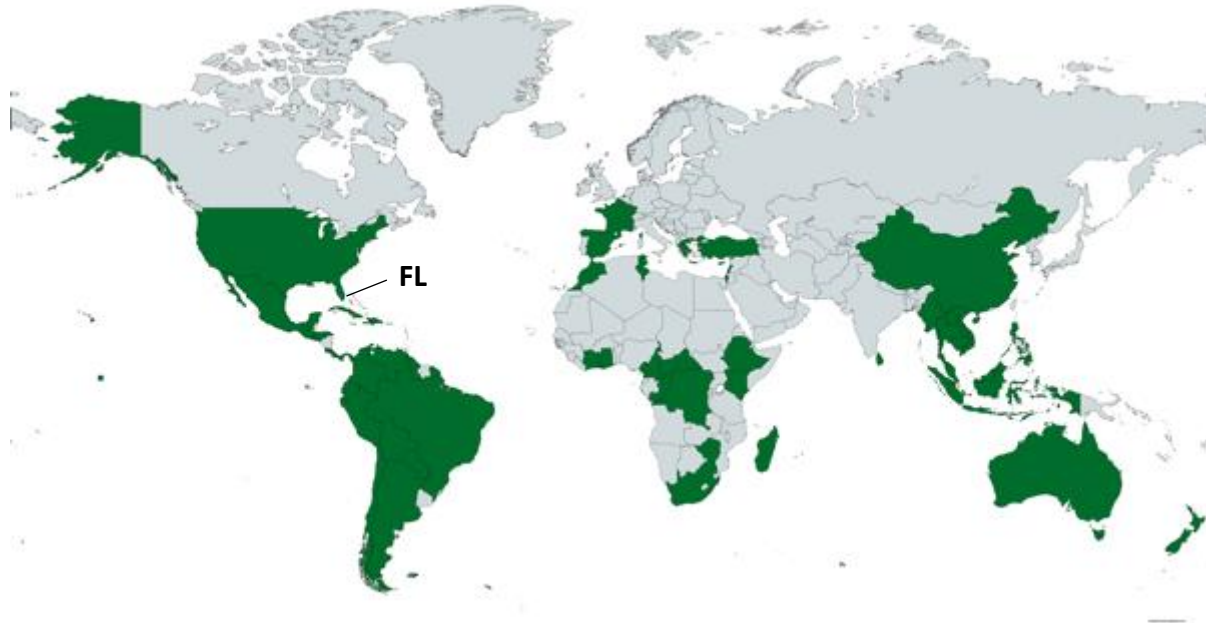


Homestead, Florida

Since it was first detected in south Florida (2012), it has killed at least 300,000 avocado trees

Distribution

Avocado Producing Countries



So far, officially reported in Florida, Myanmar, and Taiwan avocado production areas

Avocado Producing Countries + Redbay Ambrosia Beetle Natural Distribution



China, Hong-Kong, India, Bangladesh, Japan, Myanmar, South Korea, Taiwan, Thailand, Vietnam

As avocado production increases in regions where the beetle is native... more areas will be affected

Avocado

Field Symptoms

Forest Lauraceae

Loss of leaf turgor
(green wilting)

Sectorial wilting
resembling drought
stress

**Rapid disease
progression**

Susceptible trees die
within a month

Evidence of beetle
boring (though not
always present)



Lancewood, FL



Sassafras, TN



Swampbay, FL



C. osmophloeum, Taiwan



External symptoms may look different but internal symptoms are very similar

Avocado

Redbay

Sassafras

Lancewood

Cinnamon



Sapwood streaking

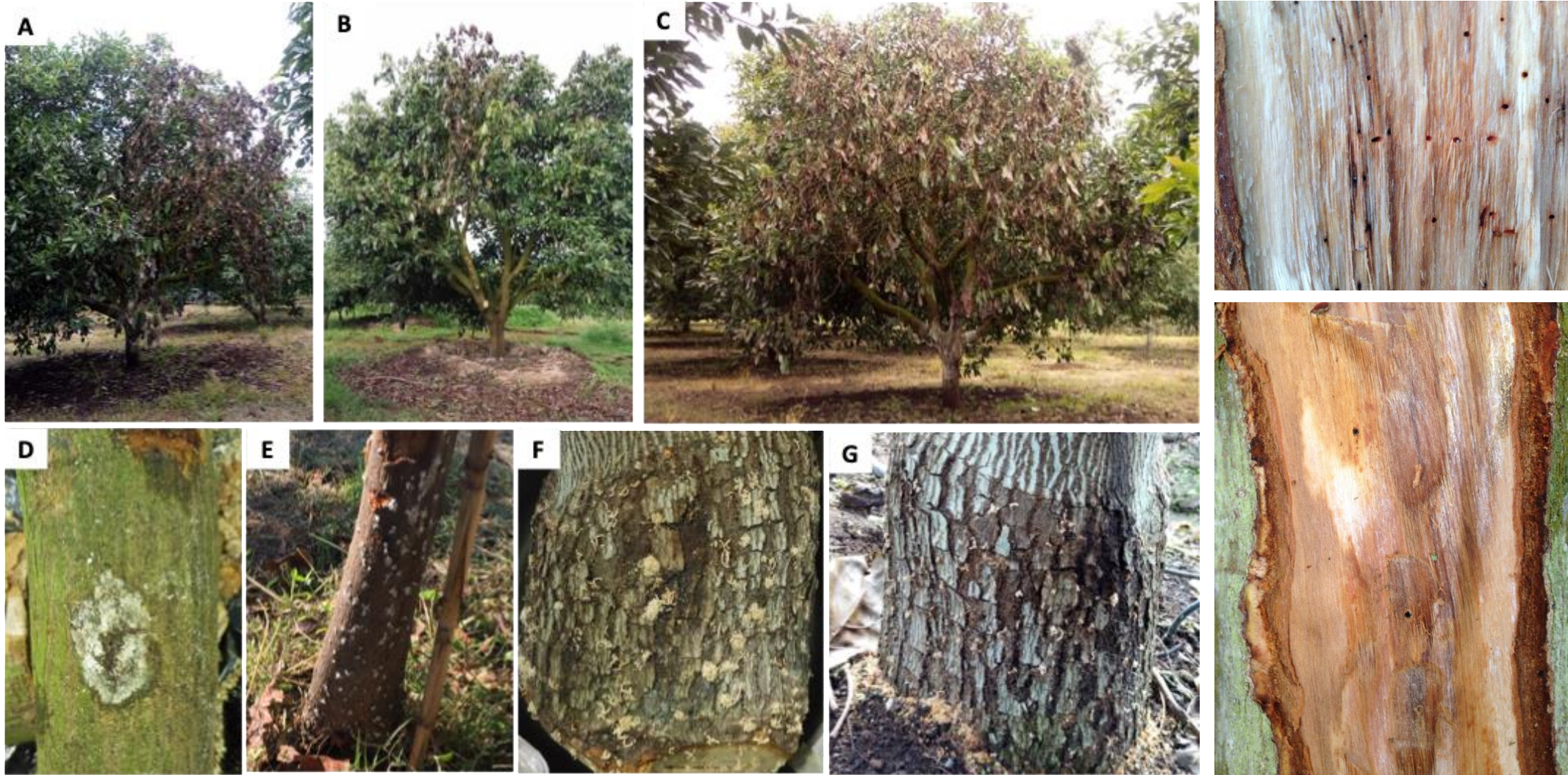
Field symptoms are not sufficient for an accurate and definitive diagnosis



Wilting backyard Avocado tree, Isabella, Galapagos, Ecuador. 2022.

Negative for Laurel Wilt

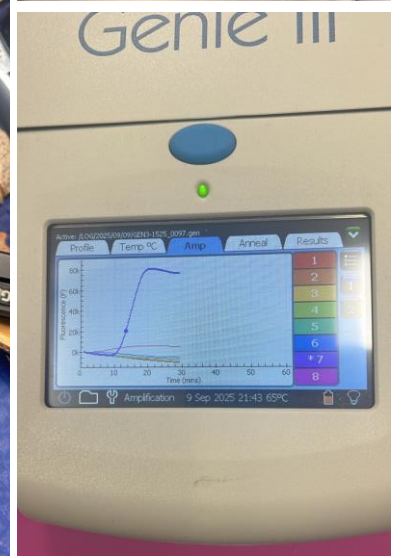
Field symptoms are not sufficient for an accurate and definitive diagnosis



Wilting Avocado trees, Mexico.
Mass attack ambrosia beetles.

Negative for Laurel Wilt

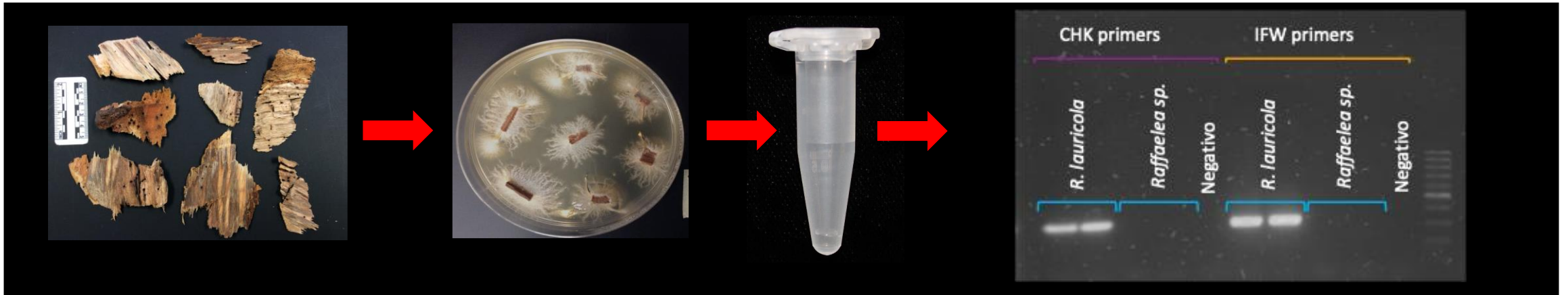
Field symptoms are not sufficient for an accurate and definitive diagnosis



Wilting Avocado trees, CA. Samples received 09/08/25.

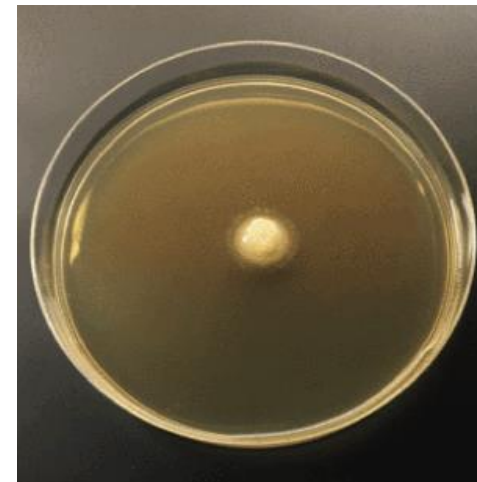
Negative for Laurel Wilt

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



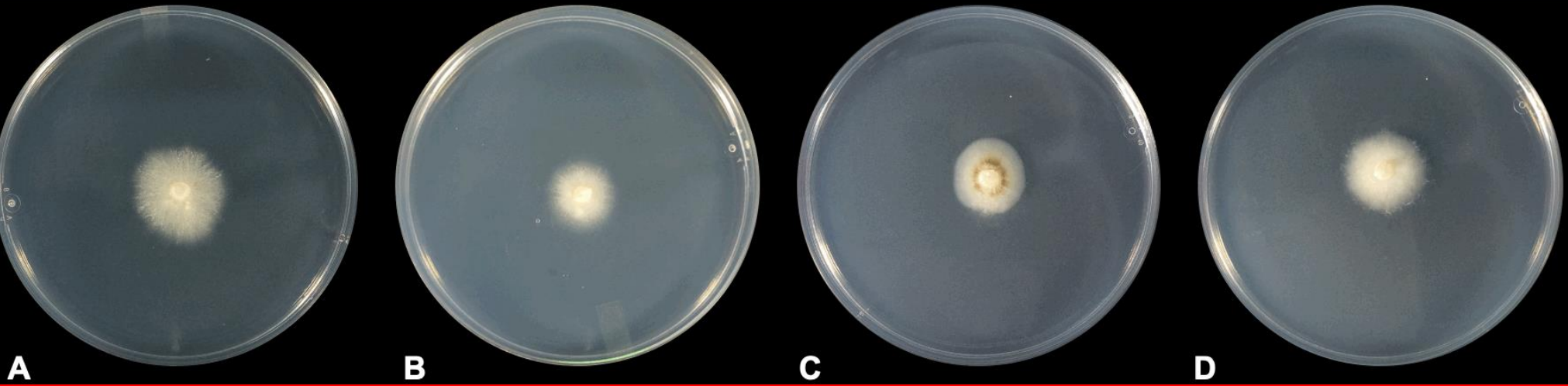
Plating in the semi-selective media
cycloheximide-amended medium (CSMA) ->
fungal growth is observed after 7-10 days ->
DNA is extracted and two primer sets are
tested IFW and CHK (Dreaden et al. 2014) OR
LSU + sequencing.

Morphology is not diagnostic



**Morphology
is not
diagnostic**

HL



Non-HL

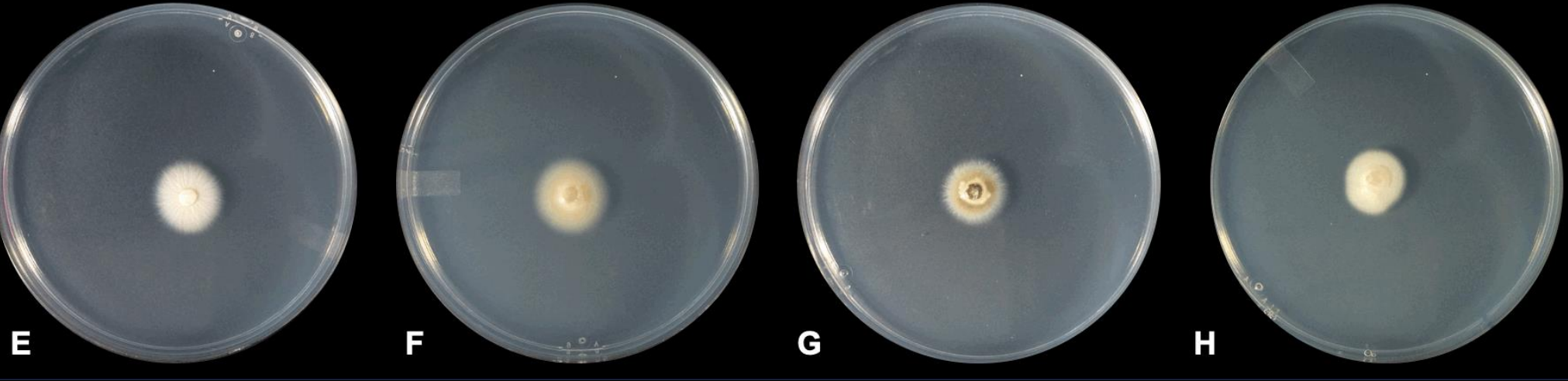
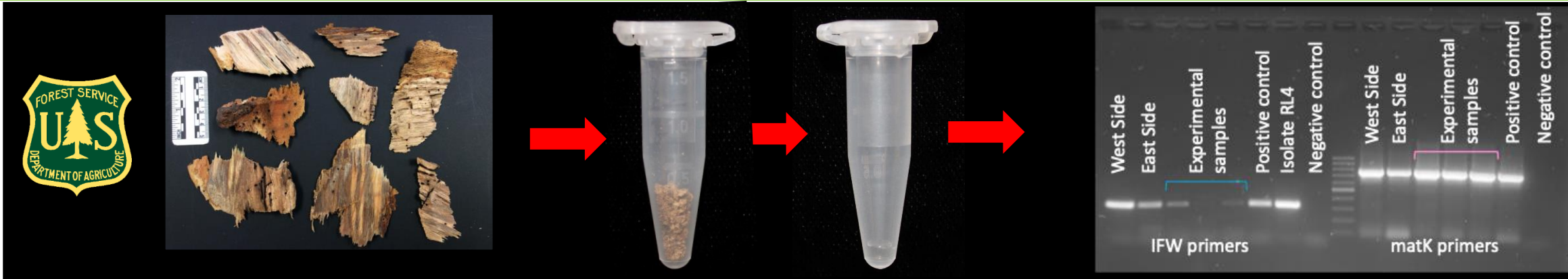


Figure 1. Seven-day old cultures of *Harringtonia lauricola* strains and fungal species with similar colony phenotype growing on CSMA. A. *Harringtonia lauricola* RL4 from *Persea americana* (Florida); B. *H. lauricola* from *Sassafras albidum* (Tennessee); C. *H. lauricola* from *P. americana* (Myanmar) D. *H. lauricola* from *P. americana* (Myanmar). E. *Hawksworthiomyces* sp. from *P. americana* (Florida). F. *H. aguacate* from *P. americana* (Florida). G. *Raffaelea* aff. *cyclorhipidia* from *Xyleborus ferrugineus* (Florida). H. *Raffaelea* aff. *ambrosiae* from *P. americana* (Florida).

Molecular Detection Protocol (trunk tissue from symptomatic trees) = 1 day



Primer development: Dreaden et al. 2014. Protocol development: Parra et al. 2020.

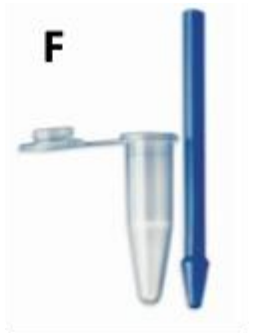
- QIAGEN Fast DNA Stool Kit (modifications). Kit has anti-inhibitors.
- IFW primer set.
- ~90% efficiency (~10% false negatives).
- No cross reactions (false positives).



- Homogenization is critical.
- Longer step is the incubation overnight.



New LAMP-based assay

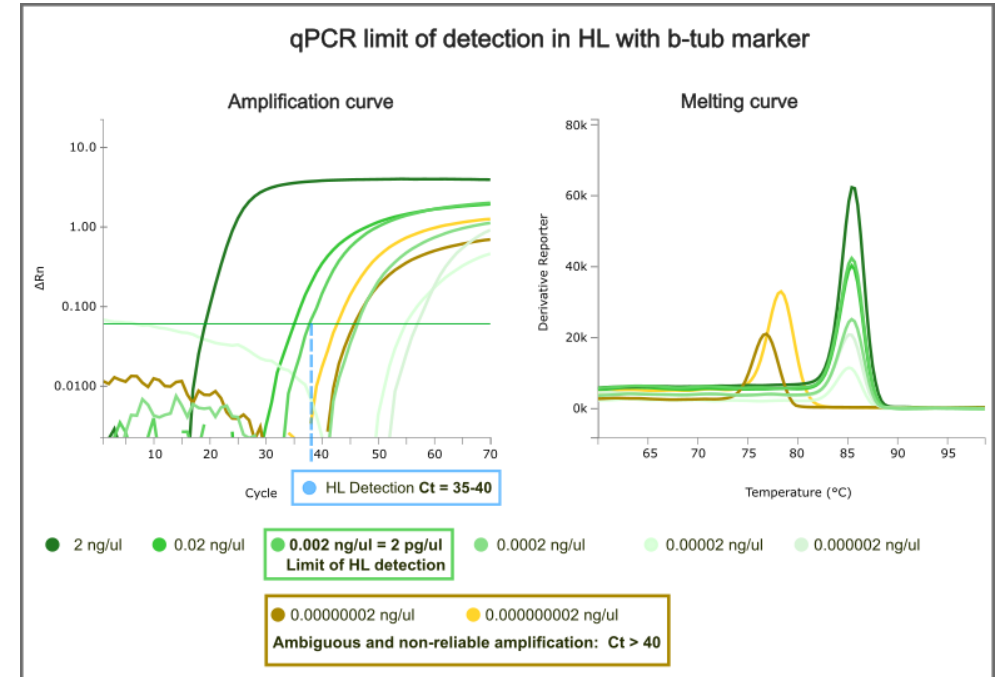
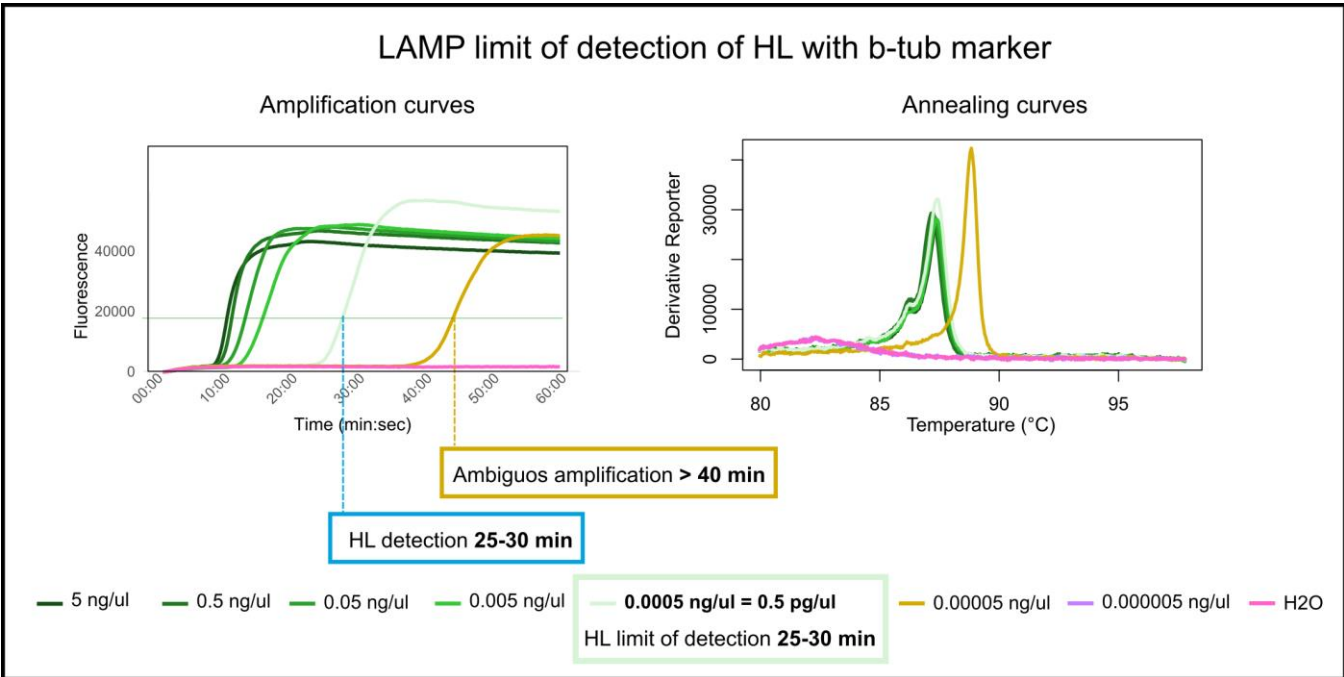


The 0354 primers are:

- HL.0354_F3: 5'-TCGCAGAGCCAGAGTTCG-3'
- HL.0354_B3: 5'-GCGCGCTGTCTTTCTGTT-3'
- HL.0354_FIP: 5' TGCGGCGGTGTTTCAGATCAGTTTTACTGGTCGTCAATGTCGCT-3'
- HL.0354_BIP: 5' TTGCCGCCTCGTGATGTTCCTTTTTGTGCTGGGCATTTTCGT-3'
- HL.0354_LF: 5'-ACTGACACCCGGACTCGATAG-3'
- HL.0354_LB: 5'-CTCAGCGCGACAGACAGTC-3'

Crude extracts of symptomatic wood and beetles

Hamilton et al. 2021 – B-tub



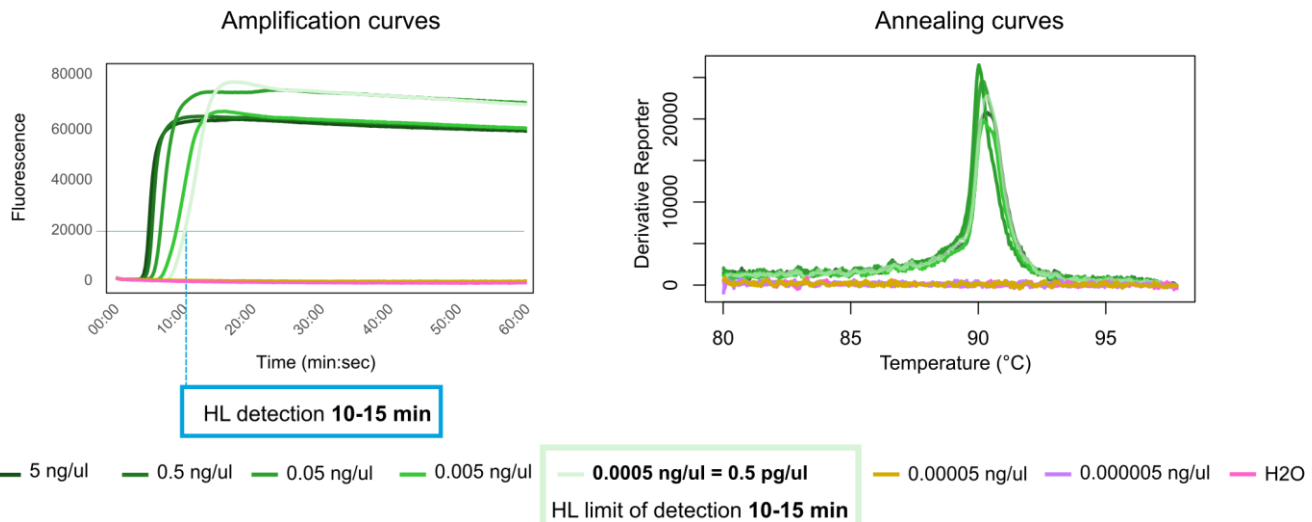
A Field-Portable Diagnostic Approach Confirms Laurel Wilt Disease Diagnosis in Minutes Instead of Days

By Jeffrey L. Hamilton, MS, Stephen W. Fraedrich, PhD, Campbell J. Nairn, PhD, Albert E. Mayfield, PhD, and Caterina Villari, PhD

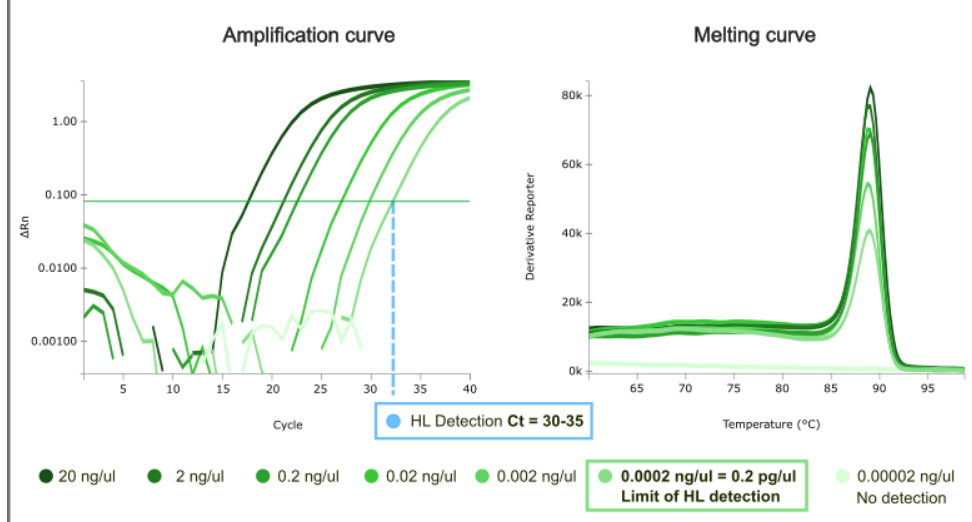
- Markers based on b-tubulin
- Validated for Avocado
- Limit of Detection HL 25 – 30 minutes and 0.5 pg/ul
- qPCR limit of detection 2 pg/ul, Ct 35-40
- Ambiguous amplification after 40 minutes and some cross-reactions and false positives

Diaz-Valderrama, Gazis, et al. 202x

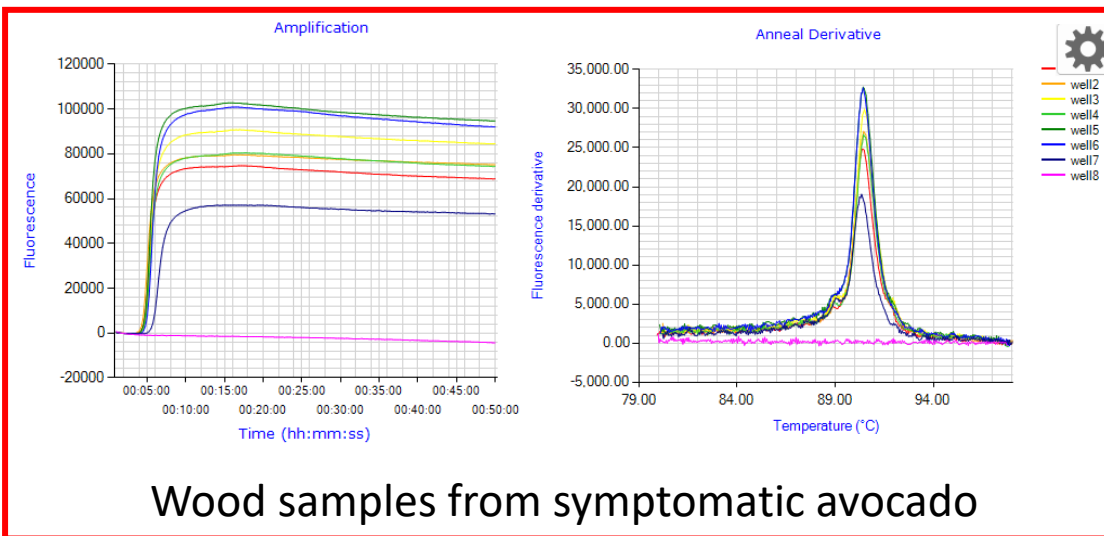
LAMP limit of detection of HL with 0354 marker



qPCR limit of detection in HL with 0354 marker



- Marker HL-354, based on 20 HL genomes
- Limit of Detection HL 10-15 minutes and 0.5 pg/ul
- qPCR limit of detection 0.2 pg/ul, Ct 30-35
- No ambiguous amplification and no cross amplification



THANK YOU! (r.gazisseregina@ufl.edu)

USDA-NIFA-SCRI award #2024-51181-43302
USDA-NIFA-SCRI award #2021-51181-35863
USDA-NIFA-SCRI award #2020-51181-32198
FDACS award #AM22SCBPFL1125
Plant Protection Act Section 7721. 2024 - 0039
Avocado Administrative Committee
USDA-IR4 program and industry partners

