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Responses of avocado to laurel wilt, caused by *Raffaelea lauricola*

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Laurel wilt, caused by *Raffaelea lauricola*, threatens native and non-native species in the Lauraceae in the south-eastern USA. Avocado, *Persea americana*, is the most important agricultural suscept of laurel wilt. Grafted plants (clonal scions on seedling rootstocks) of 24 cultivars were screened against the disease in the field from 2008 to 2010. Disease was induced with either mycelial plugs or conidial suspensions of *R. lauricola*. There were significant differences in the severity of disease that developed on different cultivars, and West Indian cultivars were most susceptible ($P < 0{\cdot}05$). Simmonds, a West Indian cultivar that comprises 35% of the commercial production in Florida, was consistently susceptible and was used as a standard genotype in different studies. Disease severity increased significantly on cv. Simmonds as plant size (stem diameter) increased ($P < 0{\cdot}0042$). In greenhouse studies, internal (sapwood) and external disease severities on cv. Simmonds were correlated ($P < 0{\cdot}0001$), and a threshold was evident, in that external symptoms developed only after moderately severe symptoms had developed internally. Latent infection was uncommon; *R. lauricola* was usually isolated on a semiselective medium or detected via qPCR only from discoloured xylem of inoculated cv. Simmonds. As basipetal movement of the pathogen was common, its movement among trees via root grafts is probable. Greater understanding is needed of the movement of *R. lauricola* in naturally and artificially infected trees, and whether sufficient tolerance exists in avocado to assist in the management of this important new disease.

Keywords: ambrosia beetle, avocado, laurel wilt, *Raffaelea lauricola*, *Xyleborus glabratus*

Introduction

In May 2002, an Asian ambrosia beetle, *Xyleborus glabratus*, was detected for the first time in the western hemisphere at Port Wentworth, Georgia, USA (Rabaglia *et al.*, 2006). Shortly afterwards, redbay, *Persea borbonia*, began to die in the surrounding area from what was eventually recognized as a new disease, laurel wilt (Fraedrich *et al.*, 2008). Early in this work, laurel wilt was associated with *X. glabratus*; a new fungus, *Raffaelea lauricola*, was shown to cause the disease, and *X. glabratus* was reported to have a symbiotic relationship with, and be the vector of, *R. lauricola* (Fraedrich *et al.*, 2008; Harrington *et al.*, 2008). These associations were unusual in that *X. glabratus* attacked healthy trees, even though ambrosia beetles typically infest dead or stressed trees (Harrington, 2005). In addition, ambrosia beetle symbionts are usually saprotrophs. Despite the tight association between *X. glabratus* and *R. lauricola* and the recovery of the pathogen from the beetle in Japan and Taiwan (Harrington *et al.*, 2011), laurel wilt has not been reported in Asia.

Due to the natural movement of *X. glabratus* and anthropogenic movement of infested debris, laurel wilt has spread rapidly along the south-eastern seaboard of the USA (USDA Forest Service, 2011). Redbay, swampbay (*P. palustris*) and other native species in the Lauraceae have been affected, and several non-native members of the family have also been affected or shown to be susceptible after artificial inoculation, including avocado, *P. americana* (Fraedrich, 2008; Mayfield *et al.*, 2008b; Smith *et al.*, 2009a,b). In the USA, laurel wilt has been reported as far west as Jackson County, Mississipi (*c.* 88·7° W) on redbay, and as far south as Miami-Dade County, Florida (*c.* 25·6° S) on swampbay (Riggins *et al.*, 2010; Ploetz *et al.*, 2011b). The latter outbreak is adjacent to Florida's primary avocado production area.

The first external, foliar symptoms of laurel wilt on avocado are wilting of terminal leaves that change from an oily green colour to brown soon after wilting (Fig. 1a). Symptoms typically develop rapidly in affected portions of the tree, but systemic development in which the whole tree dies is inconsistent (Figs. 1b,c). The production of healthy branches beneath affected regions in the tree or the

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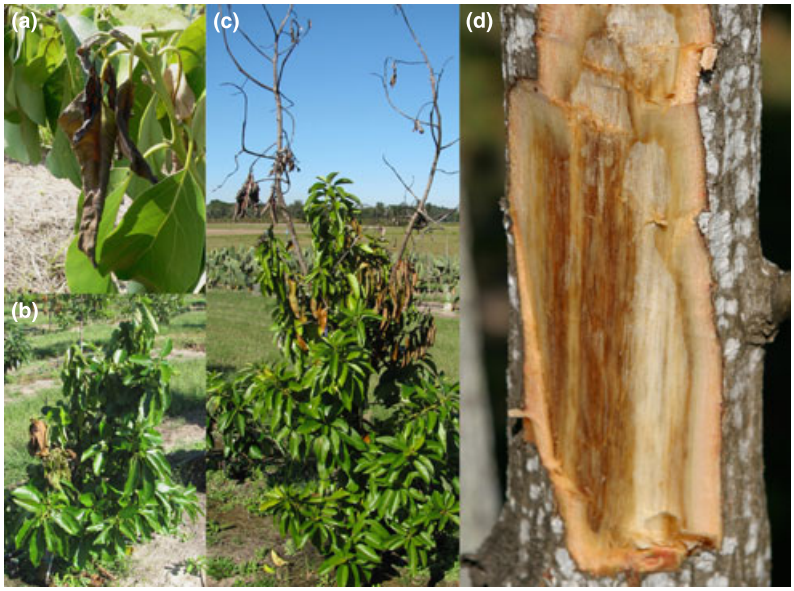

Figure 1 Symptoms of laurel wilt on cv. Simmonds avocado trees artificially inoculated with *Raffaelea lauricola* (a–c). Symptoms 10–14 days after inoculation (a), and after 1 month (b); note that symptoms are restricted to sectors above the inoculation point in b, which presumably reflect affected vascular traces. After *c.* 2 months, affected trees began to defoliate, as is evident in the upper portions of (c); note that symptoms continue to develop in this plant, but that most of the lower portions of the tree are healthy. Although some plants of susceptible cultivars died from a single inoculation, new branches often grew from beneath an inoculation point. (d) internal, sapwood symptoms in a naturally infected avocado tree.

unilateral development of symptoms in which only a branch or a portion of a tree are affected may also occur (Fig. 1c). Unlike laurel wilt-affected redbay, which retains dead leaves for a year or longer, avocado usually defoliates within 2–3 months of symptom initiation (Fig. 1c). Internally, affected avocado sapwood is discoloured reddish brown to bluish grey (Fig. 1d).

Avocado is the most important agricultural suscept of laurel wilt (Evans *et al.*, 2010; Ploetz *et al.*, 2011a). In 2006, avocado seedlings (unspecified cultivar) succumbed to artificial inoculation with *R. lauricola* in an incubator trial (Fraedrich *et al.*, 2008), and in 2007 the first naturally affected tree (unknown cultivar) was reported in the Jacksonville area (Mayfield *et al.*, 2008b). The recent outbreak of laurel wilt in Miami-Dade County suggests that it is only a matter of time before commercial avocado production is affected in Florida (Ploetz *et al.*, 2011b).

About 3·5 million metric tons (MMT) of avocado were harvested worldwide in 2008 (FAO, 2010). Mexico was the most important producing country (1·1 MMT), and the USA ranked ninth. California and Florida are the primary producing states in the USA (Evans, 2008). Commercial production in California occurs in four counties (Riverside, San Diego, Santa Barbara and Ventura), whereas that in Florida is highly localized (98% in south-eastern Miami-Dade County).

Three botanical races of avocado are recognized (Knight, 2002). The Mexican (M) (*P. americana* var. *drymifolia*) and Guatemalan (G) (*P. americana* var. *guatemalensis*) races originated in the respective highlands of those countries, whereas the West Indian (WI) (*P. americana* var. *americana*) race arose on the Pacific coast of Central America. Due to environmental adaptations and marketing histories, different cultivars are grown in California and Florida: M, G and M × G hybrid cultivars predominate in California, and WI, G and WI × G cultivars are most important in Florida (Knight, 2002; Crane *et al.*, 2007). One M × G cultivar, Hass, accounts for 95% of all production in California, but 23 major and 20 minor cultivars are produced in Florida. Commercial production in both states relies on clonal scions that are grafted on clonal or seedling (most common) rootstocks.

During the present studies, avocado responses to laurel wilt were examined after artificial inoculation with *R. lauricola*. Previous work indicated that a wide range of avocado cultivars were equally attractive to *X. glabratus* (Mayfield *et al.*, 2008a). Thus, it was assumed that results with the pathogen alone would reflect the resistance/susceptibility of the tested cultivars to natural, *X. glabratus*-mediated infection. Disease responses were determined for racially diverse cultivars, and the influence of plant size was investigated, as laurel wilt development was previously correlated with the size of redbay trees (Fraedrich *et al.*, 2008). Internal and external symptom development was examined on avocado, as was detection of the pathogen in tissue with and without symptoms. The present results will help focus future work to identify laurel wilt-tolerant genotypes of avocado.

Materials and methods

All experiments were conducted with grafted avocado plants (clonal scions on seedling rootstocks). Plants were

obtained from commercial nurseries in Florida and maintained at the University of Florida's Tropical Research and Education Center in Homestead (TREC) prior to use in an experiment. Plants were treated with standard fertilizer and irrigation practices. To manage a common problem in local nurseries, phytophthora root rot (caused by *Phytophthora cinnamomi*), plants were drenched with metalaxyl once or twice before use in an experiment. Prior work indicated that neither *R. lauricola* nor laurel wilt were affected by metalaxyl (Ploetz *et al.*, 2011c; RC Ploetz, unpublished results).

Experiments were conducted in the greenhouse and field. The Florida Department of Agriculture and Consumer Services (FDACS) allowed field studies with *R. lauricola* only in areas in Florida where this exotic pathogen had established. Thus, experiments were conducted under an FDACS permit in a locked greenhouse at TREC during 2009 and 2010. In 2007, work was conducted in a quarantine greenhouse at the FDACS facility in Gainesville, FL, but from 2008–2010 it was conducted in the field at the University of Florida's Plant Science Research and Education Unit in Citra, FL (PSREC). In the field experiments, plants were established in May or June and inoculated shortly thereafter.

Two isolates of *R. lauricola* were used to induce laurel wilt. AvoB (Mayfield *et al.*, 2008b) was used in greenhouse (2007) and field experiments at PSREC in 2008 and 2009, and RL4 (Ploetz *et al.*, 2011c) was used in the greenhouse experiments at TREC in 2009 and 2010, and field studies at PSREC in 2010. AvoB and RL4 were recovered from laurel wilt-affected avocado trees in Florida, and SSU rDNA sequences for each are deposited in GenBank under accession nos. EU123076 and HM446155, respectively. A voucher specimen of RL4, CBS 127349, is deposited at the Centraalbureau voor Schimmelcultures (CBS Fungal Biodiversity Centre, Utrecht, the Netherlands).

Two inoculation methods were used to induce laurel wilt. In 2007 and 2008, 5 mm^2 mycelial plugs from actively growing cultures on malt extract agar (MEA) were inserted in small downward flaps that were cut in the bark, 10 cm above the graft union, and sealed with Parafilm. In 2009 and 2010, 2 mm diameter holes were made with a portable drill at a 45° downward angle 10 cm above the graft union, into which was pipetted a 100 μL suspension that contained 1×10^6 conidia from MEA cultures; holes were wrapped with Parafilm after inoculation. Mock inoculations were conducted with either pieces of MEA (2007 and 2008) or water (2009 and 2010).

Cultivar experiments

A total of 24 cultivars were screened for response to laurel wilt (Table 1). Cultivars were selected based on their importance in Florida or the interest of local producers (Crane *et al.*, 2007). A few cultivars with G or M × G genomes were also tested in the interest of more broadly assessing the reaction of avocado to laurel wilt. Plants in greenhouse (2007) or field experiments (2008–2010) were transported from Homestead in moving vans immediately prior to the start of these experiments in Gainesville and Citra, respectively.

In general, the largest plants that were available from commercial nurseries in Florida were used in field experiments (all were in ≥12 L pots). In 2008 and 2009, cultivars were replicated six or 10 times, respectively, in randomized complete block designs (RCBDs), whereas cultivars were replicated 12 times in 2010. The severity of laurel wilt was assessed 1 and 2 months after inoculation on a subjective 1 to 10 scale, where 1 = no symptoms; 2 = 1–11% of the canopy showing symptoms; 3 = 12–23%; …9 = 88–99%; and 10 = dead.

Influence of plant size

The influence of plant size on laurel wilt development was assessed in three experiments with the AvoB isolate. In 2007, cv. Simmonds plants in 4 and 28 L pots were compared in the greenhouse. In the field in 2008, four sizes of cv. Simmonds plants were tested (small 12 L, large 12 L, 28 L and 60 L) (Fig. 2), and in 2009, 12 and 28 L plants of cv. Choquette and 12, 28 and 60 L plants of cv. Lula were tested. Treatments were replicated four, four and three times in RCBDs during the respective years.

Recovery of *R. lauricola*

A semiselective medium, cycloheximide + streptomycin malt extract agar (CSMA), developed by Harrington (1981), was used to assess whether or not tissue was infected by *R. lauricola* in the above experiments. In greenhouse experiments at TREC, a variant of CSMA, which contained 0·6 g cycloheximide, 0·25 g ampicillin and 0·005 rifamycin L^{-1}, was used to isolate the pathogen; hereafter, it is referred to as CSMA+. Plates were observed after 1 and 2 weeks for growth of the fungus; its characteristic phenotype on these media enabled its identification (Ploetz *et al.*, 2011b).

Host colonization and symptom development

Cultivar Simmonds plants in 12 L pots were inoculated with RL4 as described above in three greenhouse experiments; in each experiment, five plants were inoculated. After 4 weeks, plants were rated for external symptoms and bark was removed from stem and root surfaces with a knife to assess sapwood discoloration (internal symptom development). Internal symptoms were rated on a subjective scale similar to that used for external symptoms, where 1 = no symptoms; 2 = 1–11% of the sapwood showing symptoms; 3 = 12–23%; …9 = 88–99%; and 10 = 100% of tissue showing symptoms. External and internal symptom severity data from all three experiments were pooled for the regression in Figure 3.

After disease ratings were taken, tissue was removed above (at 20 cm intervals) and below (7 and 17 cm) the inoculation point, surface disinfested (20 s 70% ethanol and 2 min 10% bleach), and plated on CSMA+. To determine whether *R. lauricola* was detected reliably with CSMA+, some of the above samples were also assayed with a real-time qPCR assay (Dreaden *et al.*, 2008) (Table 2)

Table 1 Response of different avocado cultivars and genomes to laurel wilt[a]

Cultivars[b]	Genome[c]	Disease severity[d] 2008	2009	2010	Average 2008–2010	Genome average
Ettinger	G × M	n/t	2·8	n/t	2·8	2·6 c
Hass	G × M	2·8	2·7	2·7	2·7 de	
Pinkerton	G × M	n/t	n/t	3·3	3·3	
Winter Mexican	G × M	n/t	1·8	2·3	2·3 e	
Bacon	G	n/t	2·2	2	2·1 e	2·5 c
Marcus Pumpkin	G	n/t	n/t	2·3	2·3	
*Reed	G	n/t	3·5	n/t	3·5	
*Brogdon	G × M × WI	3	4·1	4·1	3·7 bcde	3·7 abc
*Beta	G × WI	n/t	3·5	4·5	4·0 abcd	3·7 b
*Choquette	G × WI	2·4	3·6	2·6	2·9 de	
*Hall	G × WI	2·3	4·9	n/t	3·9 abcde	
*Lula	G × WI	4·9	3·1	5	4·3 abc	
*Miguel	G × WI	5	3·7	n/t	4·4 abcd	
*Monroe	G × WI	5·2	2·9	3·3	3·5 cde	
*Tonnage	G × WI	n/t	3·5	3	3·3 cde	
Bernecker	WI	4·2	4·2	3·8	4·1 abcd	4·4 a
Catalina	WI	3·8	5·4	3·5	4·2 abcd	
Day	WI	n/t	4·3	n/t	4·3	
*Donnie	WI	5·4	4·5	5·4	5·1 ab	
*Pollack	WI	n/t	3·7	n/t	3·7	
*Russell	WI	n/t	5·6	5·1	5·3 ab	
*Simmonds	WI	5·3	5·8	5·8	5·6 a	
Trapp	WI	n/t	3·3	n/t	3·3	
*Waldin	WI	n/t	4·3	n/t	4·3	

[a]Large potted plants (12 to 60 L pots) were planted and inoculated with *Raffaelea lauricola* in 2008, 2009 and 2010. Results are from replicated field experiments conducted at the University of Florida's Plant Science Research and Education Center, Citra, Florida.
[b]Cultivars are either recommended for use in Florida (Crane *et al.*, 2007) and marked with an *, were suggested by local avocado producers, or were tested to more broadly assess the reaction of avocado to laurel wilt. For example, no G × M cultivars are grown commercially in Florida. Note the prevalence of G × WI and WI cultivars among those that are recommended for the state.
[c]Genome indicates whether a cultivar has a pure Guatemalan (G) (*Persea americana* var. *guatemalensis*) or West Indian (WI) (*P. americana* var. *americana*) background, or whether it is a G × WI hybrid, G × Mexican (M) (*P. americana* var. *drymifolia*) hybrid, or a complex G × M × WI hybrid (Schnell *et al.*, 2003).
[d]Average external disease severity, rated on a 1–10 subjective scale (see text). Disease severity among cultivars that were tested at least twice and genome (responses for all cultivars, regardless of the number of times it was tested) were compared using Wilcoxon non-parametric comparisons of means; means followed by the same letter are not significantly different, $P < 0.05$.

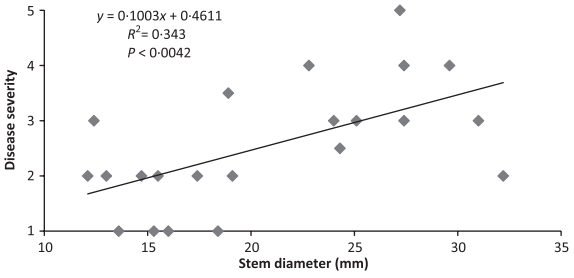

Figure 2 Relationship between stem diameter of cv. Simmonds and the severity of laurel wilt that developed on trees inoculated with the AvoB isolate of *Raffealea lauricola*. Results are from an RCB field experiment in 2008.

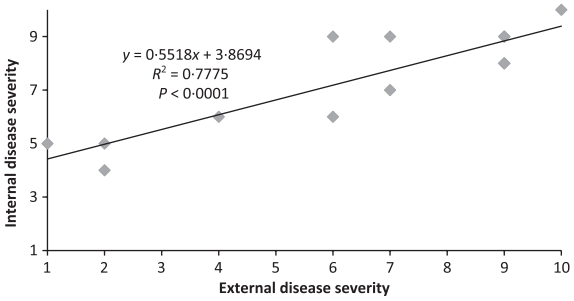

Figure 3 Correlation of external and internal symptoms of laurel wilt that developed on cv. Simmonds plants inoculated with the RL4 isolate of *Raffaelea lauricola* in three greenhouse experiments in 2009 and 2010.

using primers LWD3 5′-AACGCGTCAAAAGACAA CAG-3′ and LWD4 5′-TTTCTAGGACCGCCGTAATG-3′, which amplified a partial sequence of SSU rDNA of *R. lauricola*. A serial dilution (1 to 1×10^{-5} ng μL^{-1}) of DNA from isolate RL4, grown on potato dextrose agar (PDA), was used to construct a standard curve to determine the limit of detection for the assay and to quantify amplification results.

Table 2 Recovery and detection of *Raffaelea lauricola* from symptomatic and symptomless avocado tissue[a]

Location[b]	Internal symptoms[c]		Recovery on CSMA+[d]		qPCR (ng μL^{-1})[e]
120	Yes	1/1	Yes	1/1	n/t
100	Yes	4/4	Yes	4/4	1 (0·0014)
80	Yes	9/9	Yes	9/9	2 (0·0001, 0·0083)
60	Yes	14/14	Yes	14/14	2 (0·0002, 0·0165)
40	Yes	14/14	Yes	14/14	2 (0·0003, 0·0047)
20	Yes	14/14	Yes	14/14	2 (0·0052, 0·0061)
−7	Yes	13/14	Yes	13/14	2 (0·0002, 0·0119)
−7	No	1/14	Yes	1/1	n/t
−17	Yes	8/14	Yes	8/8	1 (0·0023)
−17	No	6/14	Yes	2/6	n/t
			No	4/6	1 (<0·0001)

[a]Data are from three greenhouse experiments with cv. Simmonds, 4 weeks after plants were inoculated with *R. lauricola*. Bark was removed from plants prior to assessing internal symptom development and assaying for the pathogen.
[b]Location = cm above (+) or below (−) the inoculation point from which a given sample was taken.
[c]Symptoms = whether or not vascular discoloration occurred at a given location, and the ratios of plants with or without symptoms. For example, one and six plants were symptomless at, respectively, −7 and −17 cm, whereas all other plants and locations showed symptoms. A total of 14 plants were assayed. As plants varied in height, samples from only some could be obtained from ≥80 cm.
[d]For each tissue sample, six 5 mm^2 pieces of surface-disinfested wood were plated on CSMA+, a semiselective medium for *R. lauricola*, as described in the text.
[e]Tissue from some samples were assayed with real-time qPCR, using diagnostic SSU rDNA primers (Dreaden *et al.*, 2008). Numbers in parentheses are ng of target DNA that were detected in a sample.

Statistical analyses

Wilcoxon non-parametric comparisons were used to separate mean disease severities for cultivars that were tested at least twice during the 2008 to 2010 field experiments, as well as mean responses for cultivar genomes; in the latter analyses, all cultivar means were used, regardless of how many times a cultivar was tested. The GLM procedure in SAS was used for regressions in Figures 2 and 3.

Results

In general, symptoms began to develop within 10–14 days of inoculation, regardless of whether mycelia or conidia were used to inoculate plants and whether plants were screened in greenhouse or field experiments. The first symptoms that were observed externally were wilted leaves and stems in branch terminals above inoculation sites that soon became greyish and then brown as tissue died (Fig. 1a). Often only a portion of the tree canopy was affected (Fig. 1b). Affected leaves became brown and abscised, usually within 4–6 weeks (Fig. 1c), and if plants did not die, branches often grew from beneath the inoculation site (Fig. 1c).

The pathogen was always recovered from sapwood with symptoms on CSMA and CSMA+ (Table 2). Symptoms of laurel wilt did not develop on mock-inoculated plants, and the pathogen was never recovered from these plants. Freezing temperatures at the PSREC field site during the winters after the 2008, 2009 and 2010 field seasons killed plants; thus, it was not possible to follow recovery and/or the continued development of laurel wilt symptoms on these plants for more than 4–5 months after inoculation.

Cultivar experiments

Availability of plants limited the testing of the following cultivars to a single year: Day, Ettinger, Marcus Pumpkin, Pinkerton, Pollack, Reed, Trapp and Waldin. However, because there was no cultivar × year interaction, cultivars that were tested during 2 or 3 years could be compared.

Unfortunately, many of the WI and G × WI cultivars that are important in South Florida were moderately susceptible to laurel wilt. Simmonds, a WI cultivar that comprises *c.* 35% of the commercial production in South Florida, was consistently among the most susceptible cultivars in these tests (Table 1). Overall, WI cultivars developed more severe symptoms than the G, G × M and G × WI cultivars that were tested ($P < 0{\cdot}05$) (Table 1).

Influence of plant size

Symptom severity was significantly ($P < 0{\cdot}05$) and positively correlated with plant size on cvs Simmonds (Fig. 2), Choquette and Lula (data not shown). Small (4 L), newly grafted plants (≤1 cm diameter) of cvs Brogdon, Reed and Simmonds (Table 1) and Choquette, Donnie, Hass, Lula, Monroe and Simmonds developed little disease after inoculation, regardless of genotype (data not shown).

Recovery of *R. lauricola*

Raffaelea lauricola was recovered on both CSMA and CSMA+ from naturally or artificially affected plants showing symptoms. Although the increased cycloheximide concentration and additional antibiotics in CSMA+ resulted in cleaner isolations (reduced recovery of other fungal and bacterial contaminants), useful data were obtained with either medium.

Host colonization and symptom development

Disease developed fairly rapidly on cv. Simmonds, with most plants developing visible external symptoms within 2 weeks of inoculation. Internally, symptoms had developed to the top of all plants after 4 weeks. Internal symptoms were conspicuous, dark brown streaks or patches that contrasted with healthy, white sapwood. Internal and external symptom severities were significantly correlated ($P < 0{\cdot}0001$), but some plants with severe vascular discoloration developed minor external symptoms (Fig. 3).

The pathogen was always recovered from vascular tissue showing symptoms on CSMA+. In only three cases was the pathogen recovered on CSMA+ from symptomless tissue, and only once was it detected with qPCR from symptomless tissue from which the pathogen was not recovered on CSMA+ (Table 2). The qPCR primers amplified a single 232 bp product with a lower limit of detection of 1×10^{-5} ng per PCR reaction. In general, low levels of fungal DNA were detected in tissues with symptoms and, rarely, in symptomless tissue by qPCR (Table 2). Target DNA was not amplified from mock-inoculated control samples.

In no case was the pathogen not detected with qPCR when it was recovered on CSMA+. Acropetal portions of plants were thoroughly colonized by *R. lauricola* after 4 weeks (recovered from all plants and locations above the inoculation point), and in only one case was the pathogen not recovered on CSMA+ or detected with qPCR beneath the inoculation point.

Discussion

Several diseases are capable of killing avocado trees (Menge & Ploetz, 2003), but none develop as quickly as laurel wilt. When considering the avocado industry in Florida, Evans *et al.* (2010) determined that losses could potentially range from $27–54 million if effective control measures were not available by the time the disease moved into the state's commercial production areas.

In less than a decade, laurel wilt has spread throughout much of the coastal plain of the south-eastern USA and as far west as southern Mississippi (USDA Forest Service, 2011). Several factors have been responsible for this rapid movement, including the prevalence in the region of highly susceptible native hosts (e.g. redbay and swampbay), effective natural spread of the pathogen via *X. glabratus*, and the anthropogenic dissemination of infested debris to distant, unaffected areas (a 550 km jump in the distribution of laurel wilt was evident in the recent report from Mississippi (Riggins *et al.*, 2010)). Although laurel wilt will continue to spread in the south-eastern USA, it is unclear how the disease will progress where avocado is more prevalent than native hosts (e.g. south-eastern Florida) or where susceptible species are uncommon (e.g. to the west of the Mississippi site in western Louisiana and Texas). Clearly, the movement of laurel wilt in the latter areas will affect the disease's spread into Mexico, California and other avocado-producing areas. To mitigate the threat it poses in these areas, better understanding is needed on the epidemiology and control of this disease (Ploetz *et al.*, 2011a).

Managing laurel wilt on avocado may require a holistic approach that incorporates diverse strategies. These may include the use of fungicides, although the efficacy and economics of the measures that have been tested to date will make this difficult in commercial production (Ploetz *et al.*, 2011c). Holistic management would also consider sanitation (destruction of trees infested with *X. glabratus* before infectious new generations of the beetle fly to healthy trees); vector management with insecticides, attractants and/or repellents; severing root grafts in affected orchards; and the use of tolerant genotypes if they were available (Peña *et al.*, 2011; Ploetz *et al.*, 2011a).

Raffaelea lauricola and its vector, *X. glabratus*, are Asian endemics that evolved with Asian members of the Lauraceae. Thus, one might expect resistance to this disease among the Asian taxa (Ploetz, 2007). The fact that laurel wilt has not been reported in Asia may indicate that members of this family in Asia either tolerate infection or are not attractive to the beetle when healthy.

Conversely, New World members of the Lauraceae have had no co-evolved history with the pathogen. As 'new encounter' hosts often possess poor or no tolerance to a given disease, good tolerance in avocado to laurel wilt may be uncommon. Additional avocado genotypes will be screened for laurel wilt tolerance in the future, in particular those with M and G backgrounds. Although few such cultivars have been assayed to date, the present results indicate that tolerance may be found in these genomes.

Two similar diseases of Asian oaks, *Quercus* spp., may also fit the new-encounter model. Japanese oak wilt (JOW) is caused by *R. quercivora*, which is a symbiont of the ambrosia beetle vector of this disease, *Platypus quercivorus*; *R. quercivora* is found in *P. quercivorus* elsewhere in its Asian range, but JOW is not (Kinuura & Kobayashi, 2006; Takahashi *et al.*, 2010; Naoto Kamata, Department of Biology, Kanazawa University, Kanazawa, Ishikawa, Japan, personal communication). Also, Korean oak wilt is caused by *R. querci-mongilicae*, a symbiont of the ambrosia beetle vector of this disease, *P. koryoensis*; *P. koryoensis* is found in Russia, and the Korean oak wilt epidemic has progressed down the Korean peninsula from the north (Kim *et al.*, 2009a,b; Naoto Kamata, personal communication). The relatively recent history of these diseases (1934 in Japan and 2004 in Korea), the susceptibility of local *Quercus* spp., and the apparent absence of the diseases in other locations where *P. quercivorus* and *P. koryoensis* are found suggest that these are also new-encounter diseases in which native, but not exotic species tolerate infection by the associated ambrosia beetle symbionts.

Results from the above studies provide new information on the avocado × *R. lauricola* interaction. Based on the present results, relatively large avocado plants are needed to determine the response of a given genotype to laurel wilt. In addition, because basipetal movement of the pathogen was common in these studies, it would presumably be capable of moving to healthy trees via root grafts. Root grafting in avocado orchards is common, and similar pathogens,

such as *Ophiostoma novo-ulmi*, cause of Dutch elm disease, move via root grafts (Stipes, 2000). Finally, as external symptoms of laurel wilt developed only after extensive internal symptoms had developed, it is probable that fungicides would not be effective against laurel wilt if these control measures were applied after external symptoms were apparent. Stipes (2000) indicated that fungicides were ineffective against Dutch elm disease once >20% of a tree's canopy had been killed by that disease.

The pathogens that cause the previously mentioned oak wilts in Asia, *R. quercivora* and *R. querci-mongilicae*, do not move far in naturally or artificially infected trees. In each case, mass attacks by the respective ambrosia beetle vectors are required to induce fatal xylem dysfunction and tree death (Kim *et al.*, 2009a,b; Takahashi *et al.*, 2010). Despite similarities between these diseases and laurel wilt, *R. lauricola* is clearly more aggressive on its American lauraceous hosts than are *R. quercivora* and *R. querci-mongilicae* on the susceptible Asian oaks. Fraedrich *et al.* (2008) caused extensive damage and killed several species in the Lauraceae after inoculating plants a single time. And in the present work, a single inoculation resulted in extensive colonization and symptom development on most of the avocado cultivars that were tested. Understanding infection and disease development in naturally affected avocado, and determining the extent to which results with artificially inoculated potted plants relate to what would be expected in naturally affected field-grown trees will be critical when developing management strategies for laurel wilt on this crop.

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