Laurel wilt: A global threat to avocado production

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Abstract. Laurel wilt kills members of the Lauraceae plant family, including avocado. The disease has invaded much of the southeastern USA, and threatens avocado commerce and homeowner production in Florida, valuable germplasm in Miami (USDA-ARS), and major production and germplasm in California and MesoAmerica.

Laurel wilt is caused by a recently described fungus, *Raffaelela lauricola*, which is vectored by an invasive ambrosia beetle, *Xyleborus glabratus*. Current research topics include: identifying host resistance; disease management with fungicides; vector mitigation with insecticides and repellents; and host:pathogen interactions. Although most genotypes of avocado that have been tested are susceptible, ongoing work investigates tolerance that has been evident in Guatemalan and Mexican backgrounds. Effective fungicides have been identified, but cost-effective disease management will depend on improved measures for xylem loading and enhanced retention of these chemicals, as the protective levels of the most effective compounds, the triazoles, are retained for only 1 year. Insecticides have been identified that reduce boring activity of *X. glabratus* and its attraction to avocado and other hosts, but much remains to be learned about their impact on disease management. *Raffaelela lauricola* rapidly colonizes avocado after infection, but to low titers; the pathogen is scarcely evident in histological examinations of infected plants. Nonetheless, rapid reductions in xylem function and hydraulic conductivity occur before the development of symptoms of laurel wilt. By the time external symptoms develop in avocado, it may be difficult to manage laurel wilt. Better understandings of the temporal and spatial development of infection and how the host responds to infection may assist management efforts and the selection of laurel wilt-tolerant avocado cultivars.

Secamiento del Laurel: Una amenaza global para la producción de aguacate
El secamiento del laurel afecta miembros de la familia Lauraceae, lo cual incluye al aguacate.Esta enfermedad ha invadido la region suroriental de los Estados Unidos, y amenaza los aguacates de Florida que estan en produccion comercial, en huertas caseras y a la coleccion de germoplasma de aguacate del USDA-ARS en Miami, a la produccion de aguacate en California y en MesoAmerica.

El secamiento del laurel es causado por *Raffaelea lauricola,* un hongo que fué descrito recientemente. El vector de este hongo es un cucarroncito de ambrosia invasor,*Xyleborus glabratus*. Los tópicos de investigación actual con este problema incluyen: el manejo de la enfermedad con fungicidas; la identificacion de resistencia en hospederos; el manejo del vector con insecticidas y repelentes; los rangos de hospederos y las interacciones entre el patógeno y el vector; la transmisión de *R. lauricola* por semillas y estacas utilizadas para acodo de aguacate, los patrones radiculares y las herramientas de sesgo.

Aunque no se han identificado todavia variedades de aguacate altamente resistentes a la enfermedad, se esta continuando con un trabajo de evaluación de nuevos cultivares y evaluación de nuevas fuentes de germoplasma. Se han identificado fungicidas efectivos (e.g., triazoles), pero el manejo de la enfermedad a un costo efectivo, va a depender de tecnicas que mejoren la absorción de estos productos por el xilema y de su retención dentro del arbol. Se han determinado insecticidas que reducen la actividad de perforación de *X. glabratus* y de otros escolítidos en aguacate así como también la atraccion de *X. glabratus* a aguacate y a otros hospederos. Aunque el rango de hospederos de la enfermedad esta restringido a las especies Americanas de la familia Lauraceae, se conoce que no-hospederos también atraen a este insecto. Después de la infección, *Raffaelea lauricola* coloniza rapidamente al aguacate, pero esto es en niveles muy bajos. La inducción de tylose y gel en el hospedero se asocian con mas el impedimento de transporte de agua y el desarrollo de síntomas, que con la obstrucción del xilema por la biomasa del hongo. Las semillas y las frutas de aguacate no parecen estar infectadas por *R. lauricola.*

Keywords: *Raffaelea lauricola, Xyleborus glabratus*, redbay ambrosia beetle, avocado, *Persea americana*, quarantine, detection, sanitation, eradication

Introduction

Laurel wilt is a lethal disease of avocado, *Persea americana* Mill. (Lauraceae, Laurales, Magnoliid complex) (Fraedrich et al. 2008). It is caused by *Raffaelea lauricola* T.C Harr., Fraedrich & Aghayeva, an asexual ascomycete that is a symbiont of an Asian ambrosia beetle, *Xyleborus glabratus* Eichhoff, the pathogen's vector (Harrington et al. 2008). *Xyleborus glabratus* was first detected in the Western Hemisphere in May 2002 at a seaport near Savannah, Georgia (Rabaglia et al. 2006).

Ambrosia beetles typically infest dead or stressed trees in which they establish gardens of ambrosia fungi (Harrington 2005). These fungi are coevolved symbionts of the beetles and are usually saprobes. They are carried in specialized structures in the insects, mycangia, and are the insect's sole or primary food source. The beetles do not consume wood but, rather, cultivate and consume lawns of these symbionts in their natal galleries.

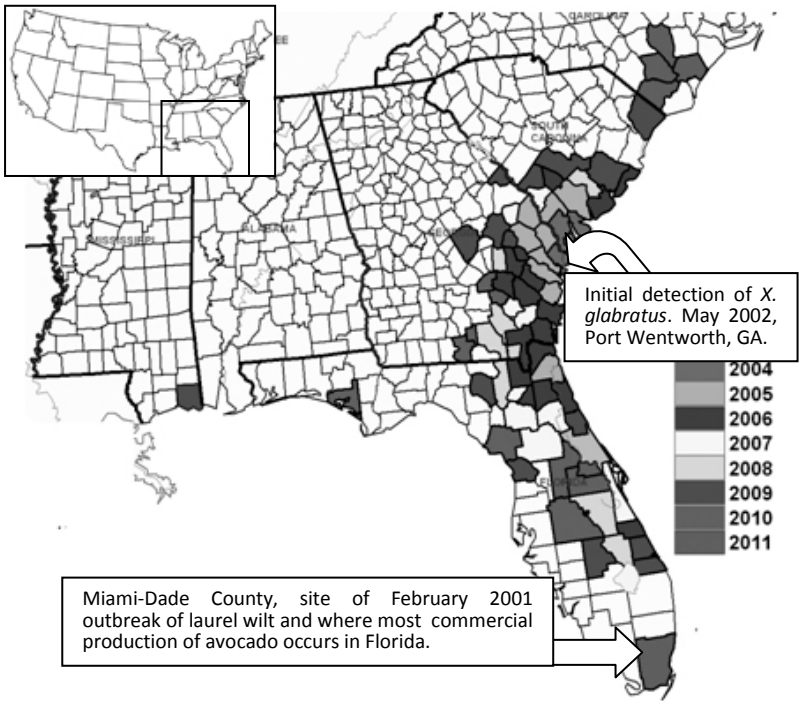

Figure 1. Distribution of laurel wilt, April, 2011 (USDA 2011).

Laurel wilt is unusual, in that *X. glabratus* attacks healthy trees and its fungal symbiont, *R. lauricola*, is a virulent pathogen. The tight association between ambrosia beetles and their fungal symbionts, and the recent report of *R. lauricola* in individuals of *X. glabratus* from Asia suggest that the pathogen entered the USA with *X. glabratus* in 2002, even though laurel wilt has not been reported in Asia (Harrington et al. 2011).

In less than a decade, laurel wilt has spread throughout much of the southeastern Atlantic coastal plain (Fig. 1). As of April 2011, the disease had been recognized as far north in the USA as Sampson County, North Carolina (35^{o} N), as far south as Miami-Dade County, Florida (25.7^{o} N), and as far west as Jackson County, MS (ca. 88.7^{o} W) (Ploetz et al. 2011b, Riggins et al. 2010, USDA Forest Service 2011). The Miami-Dade outbreak was found on swampbay, *P. palustris* (Raf.) Sarg., and is 6 km to the north of Florida's primary commercial production areas for avocado (Ploetz et al. 2011b). Possible economic losses to Florida's avocado industry have been estimated between $27 and $54 million (Evans et al. 2010).

Laurel wilt affects several species in the Lauraceae plant family (Fraedrich et al. 2008). Its rapid movement in the southeastern USA has been due to its efficient insect vector, the widespread distribution of highly susceptible native *Persea* spp., most notably *P. borbonia* L. (Spreng.) (redbay) and *P. palustris*, and the anthropogenic dissemination of infested materials. For example, a 100-km jump occurred after a hobbyist transported laurel wilt-affected wood from Jacksonville to Volusia County, Florida, and a 550 km-jump to Mississippi probably involved similar activity (Chemically Speaking 2009, Hughes unpublished data, Riggins et al. 2010).

Much remains to be learned about the relative susceptibilities to laurel wilt of native and non-native hosts in the southeastern USA and their attractiveness to *X. glabratus*. The abundance and distribution of these taxa plays a presumed, but insufficiently studied, role in the epidemiology of this disease (Koch and Smith 2009). Understanding these relationships will be important as the disease moves in the USA and if it is found in another avocado-producing country.

Laurel wilt is an immediate threat to commercial avocado production in Florida, centered in Miami-Dade County (Fig. 1), as well as the National Germplasm Repository for avocado in Miami (USDA-ARS). Elsewhere, major production throughout the Western Hemisphere, which includes seven of the world's top 10 producers (FAOSTAT 2010), is at risk.

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 Jacksonville, Florida (Mayfield et al. 2008c). Residential avocado trees have continued to die as the disease moved south in the state but, as of June 2011, Florida's commercial avocado-production area had not been affected.

About 3.5 million metric tons (MMT) of avocado were harvested worldwide in 2008 (FAOSTAT 2010). Mexico was the most important producer, whereas the USA was ranked ninth globally. California and Florida are the primary producing states in the USA.

Due to their respective environmental adaptations, historical dissemination, and local market preferences, different avocado cultivars are grown in different areas. For example, Mexican (M) (*P. americana* var. *drymifolia*), Guatemalan (G) (*P. americana* var. *guatemalensis*) and MxG hybrid cultivars predominate in Mexico and California, but West Indian (WI) (*P. americana* var. *americana*), G and WIxG cultivars are most important in Florida and the humid tropics (Crane et al. 2007, Knight 2002).

We summarize recent work to understand how avocado responds to laurel wilt, and how the disease might be managed in the future.

Materials and methods

Experiments were conducted with grafted avocado plants that are used in commercial production (clonal scions on seedling rootstocks) (Ploetz et al. 2010, 2011c). Greenhouse or field experiments were conducted, depending on where in Florida laurel wilt had been documented during a given year and the consequent restrictions that were imposed by the Florida Department of Agriculture and Consumer Services (FDACS) (field experiments with this invasive pathogen could not be conducted in the field in disease-free or newly affected areas). Greenhouse experiments were conducted in 2007 at quarantine facilities of FDACS, Division of Plant Industry in Gainesville, FL, and under FDACS permit from 2009 to 2011 in a secure greenhouse at the University of Florida's Tropical Research and Education Center in Homestead. Field experiments were conducted from 2008 to 2010 at the University of Florida's Plant Science Research and Education Unit in Citra.

To induce disease, plants were artificially inoculated with isolates of *R. lauricola*. Either patches of mycelium were inserted in clefts cut 5 cm above the graft union (2007 and 2008) or 100 μl of conidial suspensions (10^5 conidia ml^{-1}) were inserted in holes that were drilled in stems (2009-2011). Inoculation sites were wrapped in Parafilm.

Every 2-3 wks after inoculation (WAI), field experiments were rated for external disease development. In some cases, plants in the field were inoculated a second time several months after the first inoculation. Since cold temperatures killed plants at the Citra field site during the winters of 2008, 2009 and 2010, data could not be taken from those experiments the following year.

Greenhouse experiments were usually terminated 5 WAI, at which time plants were dissected to also record internal symptom severity and the linear extent of symptom development (vascular discoloration). With the exception of the 2007 and 2008 experiments, during which a 1-5 subjective disease severity scale was used, a 1-10 scale was used to rate external and internal disease development, wherein 1 = no symptoms; 2 = 1-11% of the canopy or sapwood symptomatic; 3 = 12-23%; …9 = 88-99%; and 10 = dead or completely symptomatic. Internal disease severity was assessed after bark was removed from the main stem with a knife.

The causal fungus was recovered on a semi-selective medium that was developed by Harrington (1981), CSMA. Host tissue from specific locations along inoculated stems were assayed on the medium and via qPCR to determine the extent of colonization by *R. lauricola* and the relationship between colonization and symptom development.

Cultivar experiments. *Xyleborus glabratus* bores into all avocado cultivars that have been tested (Mayfield et al. 2008b, Peña personal communication), and there is no evidence that attraction of the beetle differs among different races of avocado (Kendra et al. 2010). Thus, it is assumed that artificial inoculation with *R. lauricola* would provide useful information on how different avocado genotypes respond to natural inoculation by the beetle.

Since 2007, different avocado cultivars have been tested for response to laurel wilt in Gainesville and Citra, FL (Ploetz et al. 2010, Ploetz unpublished). In field experiments at Citra in 2008, 2009 and 2010, responses to laurel wilt were determined for 26 cultivars (Table 1). Cultivars were replicated six to ten times in randomized complete block designs, and plants were inoculated and rated for disease three (2008) or two (2009 and 2010) times. These experiments have utilized grafted trees in 28-60 L pots that have been planted/established in the ground before artificial inoculation with *R. lauricola*.

Previous observations indicated that large redbay trees develop laurel wilt symptoms more quickly and severely than smaller plants (Fraedrich et al. 2008). Thus, the influence of plant size on the development of this disease on avocado was investigated. Different sizes of 'Simmonds,' a susceptible WI cultivar, were tested in an initial greenhouse experiment in 2007 (1-gal and 7-gal pots), and a field experiment at Citra in 2008 with 15-gal, 7-gal and small and large plants in 3-gal pots. Stem diameters were recorded in the field experiment and used to assess the relationship between size and the severity of disease that developed (Fig. 2).

Since large plants are expensive and not available for many cultivars, it was also of interest to determine whether small plants would develop reliable symptoms if they were inoculated multiple times. To investigate this possibility, newly grafted plants ($\leq$ 1 cm dia) of 'Choquette' GxWI, 'Donnie' WI, 'Haas' GxM, 'Lula' GxWI, 'Monroe' GxWI, and 'Simmonds' were inoculated either one or five times.

Management with fungicides. Dutch elm disease, caused by *Ophiostoma ulmi* and *O. novo-ulmi*, and laurel wilt on redbay can be controlled with macroinfusions (injections) of Alamo, an injectable formulation of propiconazole (Mayfield et al. 2008a, Stipes 2000). To determine whether Alamo macroinfusion would be an effective and economical means for managing this disease in commercial avocado production, economic analyses were conducted for various macroinfusion scenarios and a standardized production situation in southern Florida (Ploetz et al. 2011c). Under conditions in southern Florida, macroinfusion was not cost-effective, even when a single application was presumed to efficacious for 2 or 3 years (in fact, 1 year may be more realistic for this fungicide; see Ploetz et al. 2011c).

In the interest of identifying alternative means to manage the disease, other fungicides and application measures were evaluated (Ploetz et al. 2011c). Twenty fungicides in 15 chemical groups and 10 fungicide groups were examined in vitro (Fungicide Resistance Action Committee 2010, Table 2). In general, these data were used to select products for disease suppression on artificially inoculated, potted 'Simmonds' (Table 3). Plants were treated with fungicides in one of three ways: 1) drench applications in which 1 L suspensions of fungicides were poured on the soil surface; 2) bark-directed applications in which 100 ml of fungicide suspensions were sprayed on trunk and branch surfaces in 2% Pentrabark (AGRICHEM, 5171 Morning Song Dr., Medina OH 44256); and 3) granular applications (only Prophesy, a granular formulation of propiconazole, in expt 1). After 3 wks, plants were inoculated with *R. lauricola*. Mock-inoculated plants were treated with water. After 5 wks, plants were evaluated externally and internally for disease severity. In addition, the linear extent of internal vascular discoloration, relative to the inoculation point, was recorded when internal severities were assessed.

Insecticides. Chemical control of *X. glabratus* is not viewed as a primary management strategy for laurel wilt, but may prove useful in the holistic management of this disease (Ploetz et al. 2011a). Field and laboratory tests were conducted using avocado logs, potted avocado trees, and field grown swampbay treated with contact and systemic pesticides (Peña et al., 2011).

Host:pathogen interactions. Greenhouse studies were conducted in Homestead to determine the relationship between internal and external symptom development on 'Simmonds' and colonization of the host by *R. lauricola* (Ploetz et al. 2010, Fig. 3). Symptom development was rated as above, and presence of the pathogen was assessed with CSMA and qPCR. The interaction between avocado and *R. lauricola* was also studied by examining inoculated tissue, stained for various biochemical compounds, with light microscopy.

The symptoms of laurel wilt, which include rapid wilting, necrosis of foliage and defoliation, suggest that impaired water transport may play an important role in the development of this disease. To understand more fully the impact of laurel wilt on avocado, xylem function was examined in artificially inoculated trees of 'Simmonds' (Inch and Ploetz 2011). In time course studies, water transport through infected stems was assessed under partial pressure (350 mm Hg) as ml^{-1} min^{-1} cm^{-2} (Fig. 4). In

addition, proportions of xylem that remained functional were estimated by quantifying areas that were stained by (transported) 0.1% aqueous acid fuchsin.

Results and discussion

Cultivar experiments. Overall, WI cultivars were significantly more susceptible than the G or GxM hybrids that were tested (Table 1). 'Simmonds,' a WI cultivar that comprises 35% of the commercial industry in South Florida, was consistently among the most susceptible cultivars in this work. In contrast, cultivars with M and G backgrounds generally developed less severe disease.

Ongoing work investigates resistance at the Citra field site in a more extensive collection of cultivars with M and G backgrounds. Furthermore, evaluations have begun of open-pollinated seedling progeny from the Miami USDA avocado collection at the USDA station in Ft Pierce, FL (Ploetz and Schnell, unpublished).

In experiments to assess the impact of plant size on disease development, disease severity increased significantly on 'Simmonds' as stem diameter increased (Ploetz et al. 2010; Fig. 2). Newly grafted plants (≤ 1 cm dia) of 'Choquette,' 'Donnie,' 'Hass,' 'Lula,' 'Monroe,' and 'Simmonds' developed little disease and recovered within 2 mos of inoculation, regardless of whether plants were inoculated one or five times (data not shown).

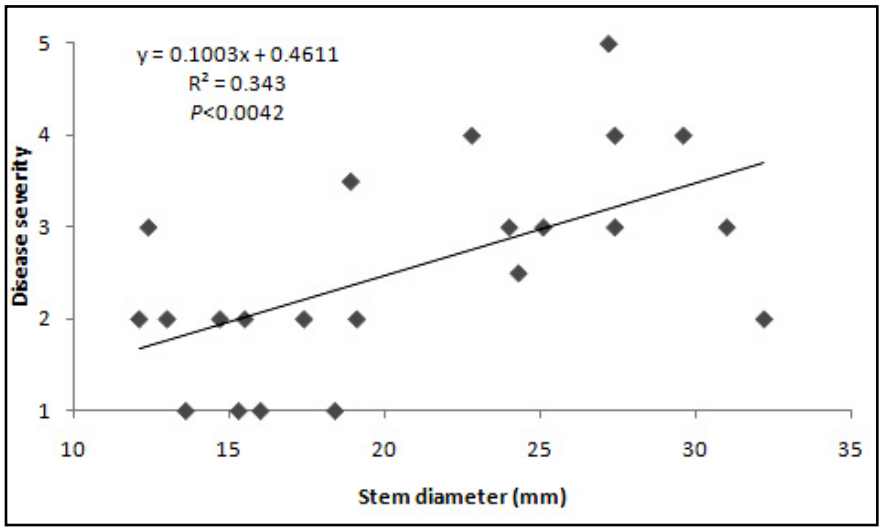

Figure 2. Relationship between plant size and severity of laurel wilt that developed on 'Simmonds' avocado artificially inoculated with *Raffaelea lauricola*

The apparent requirement for large plants in disease studies complicates the identification of resistant genotypes of avocado, as these plants are expensive and unavailable for many cultivars. These results also raise concerns about how well the above results reflect what would occur on larger trees. For example, would larger trees in commercial production be more susceptible and, if so, of what value are the above field trials?

Management with fungicides. Soil drench applications of several demethylation inhibitors (DMIs) and thiabendazole provided significant control of the disease (Table 3; $P<0.05$). DMI fungicides were equally effective, and since five of these products were triazoles, all members of that chemical group appeared to be efficacious against laurel wilt on avocado. Topical branch/trunk applications of one of the triazoles, propiconazole, in 2% Pentra-bark, a bark-penetrating surfactant, were effective at lower rates than were used in drench applications of this fungicide. Comparable levels of disease suppression were achieved when propiconazole was applied at 11% of the rates that were used in soil drenches (Ploetz et al. 2011c). Despite good *in vitro* activity against the pathogen, azoxystrobin and fluazinam had no impact on disease development in the greenhouse work. Unfortunately, Agri-fos, which is labeled for avocado and has xylem and phloem mobility, was also ineffective regardless of the way in which it was applied.

Macro-infusion (injection) has been used to effectively apply fungicides to tree vascular systems (Stennes 2000, Stipes 2000), and has been used to inject propiconazole in avocado and redbay (Mayfield et al. 2008a, Ploetz, unpublished data). Unfortunately, economic analyses indicate that macroinfusion would be too expensive for use in commercial avocado production (Ploetz et al. 2011c). Thus, other means of propiconazole application or other fungicides with a longer residual life in xylem are needed. Topical bark applications would be a less expensive practice than macroinfusion, but

moving sufficient concentrations of propiconazole or other fungicides into host xylem has been difficult in trees that are larger than the potted plants that were tested in these trials (Ploetz et al. 2011c).

Ongoing work examines means by which this goal might be met on fruit-bearing trees in the field, as well as the long-term efficacy of macroinfusion of different fungicides. For example, macroinfused thiabendazole is effective against Dutch elm disease for 3 years. Cost-effective laurel-wilt management may be possible if this fungicide is effective for as long against laurel wilt on avocado (retreatment via macroinfusion is a major expense). Fortunately, minimal fungicide has been found in fruit from trees that were treated with either propiconazole or thiabendazole, presumably due to the phloem, rather than xylem, vascular connection of these organs (Ploetz unpublished).

Insecticides. In general, zeta-cypermethrin+bifentrin and lambda-cyhalothrin+thiamethoxam provided the most consistent control of *X. glabratus*, whereas results with methomyl, malathion, bifenthrin, and endosulfan were inconsistent (Peña et al. 2011). Fewer beetles bored into avocado trees treated with fenpropathrin, cryolite Na Al fluoride, and lambda-cyhalothrin+thiametoxam than into untreated control trees. Acetamyprid+Li 100 and a mixture of imidacloprid+cyfluthrin resulted in fewer entrance holes in swampbay. Avocado logs were also baited with Beetle Block (verbenone), resulting in significantly reduced beetle emergence compared to logs that were not baited.

Research is underway to determine the potential of repellents or protectants, such as methyl jasmonate, verbenone and methyl salicylate, to prevent beetle attack of avocado trees. Volatiles from non-host plants are also being identified and tested for repellency of the redbay ambrosia beetle with the goal of identifying more effective crop protectants (Peña et al. unpublished).

Host:pathogen interactions. Internal and external disease development on 'Simmonds' were correlated (Fig. 3). A threshold for xylem dysfunction (internal symptoms) was noted; external symptoms (e.g. wilting and defoliation) developed only after relatively severe internal symptoms developed. Latent infection was uncommon, in that *R. lauricola* was isolated on CSMA only from discolored xylem of inoculated 'Simmonds', and was detected infrequently in the advance of such symptoms with qPCR (Ploetz et al. unpublished).

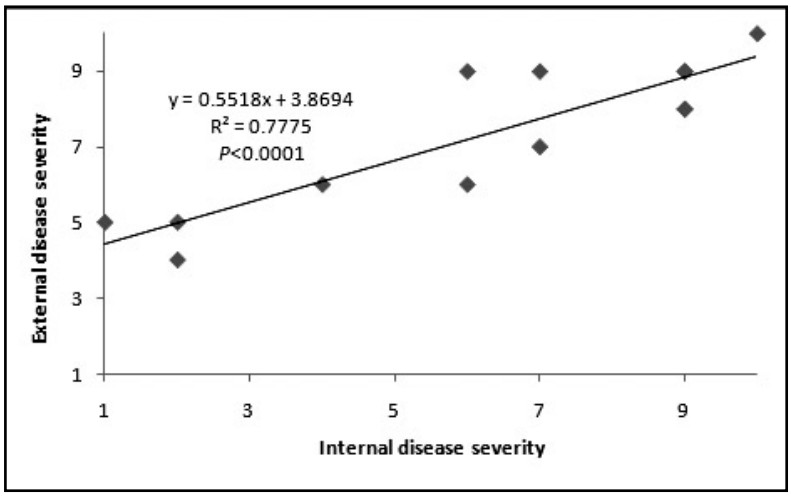

Figure 3. Relationship between internal and external development of laurel wilt on 'Simmonds' avocado artificially inoculated with Raffaelea lauricola

The pathogen rapidly colonized and affected avocado (Inch and Ploetz 2011). By 3 days after inoculation (dai), *R. lauricola* was isolated from above and below the inoculation point, and xylem function and hydraulic conductivity were significantly impaired ($P < 0.0001$; Fig. 4, and data not shown). By 7 dai, *R. lauricola* could be recovered from the entire length of some inoculated stems, often a meter or more above the inoculation point, and slight symptoms of laurel wilt had begun to develop internally and externally. However, only after profound reductions had occurred in xylem function (14 and 21 dai) did conspicuous wilting and foliar necrosis develop. By 14 dai, extensive vascular discoloration had developed and there was a dramatic reduction in functional xylem; plants with internal disease severities of 7 or greater had less than 20% functional xylem. Hydraulic conductivity decreased exponentially as nonfunctional xylem and disease severity increased. In plants with internal severities greater than 7, mean flow rates of water were 0.07 ml^{-1} min^{-1} cm^{-2} vs 42 ml^{-1} min^{-1} cm^{-2} in mock-inoculated plants.

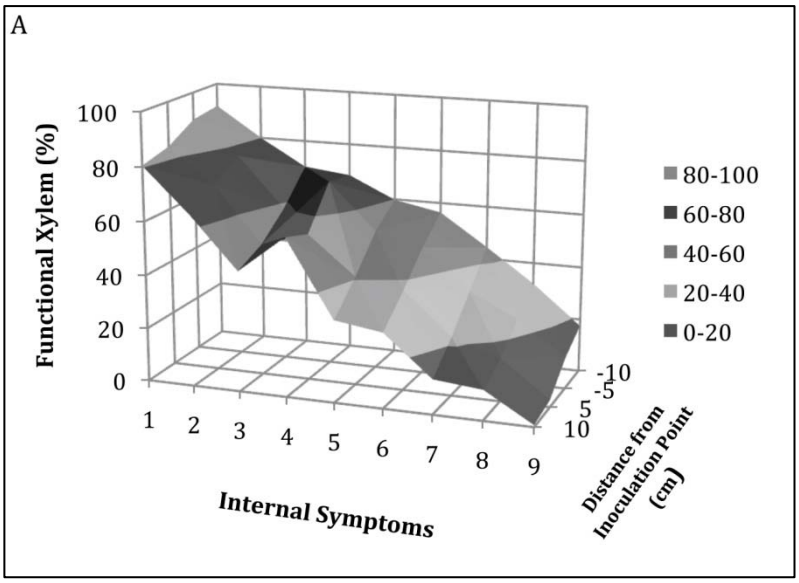

Figure 4. Relationship between internal laurel wilt severity and functional xylem at 5 and 10 cm above and below the inoculation point.

Avocado responds to infection by *R. lauricola* by accumulating phenolic substances and producing tyloses in vessel elements, typical host defense responses (Ploetz et al. 2010, Inch and Ploetz unpublished). Studies are underway to distinguish macroscropic and microscopic reactions of susceptible and tolerant cultivars of avocado and other host species against this disease.

The rapid development of these changes suggests that it may be difficult to manage laurel wilt in avocado once plants are infected by *R. lauricola*. Better understandings of the temporal and spatial development of infection and how the host responds to infection may assist efforts to select laurel wilt-tolerant avocado cultivars.

Conclusions

Cultivars of avocado with a WI pedigree (those that are important in Florida) have been most susceptible to laurel wilt in screening trials. It is hoped that tolerance that has been observed in previous trials in M and G genotypes (very few have been tested) will be evident when a more extensive collection of such cultivars are screened in 2011. Additional tolerance might also be revealed in open-pollinated seedling progeny from the USDA clonal collection of this crop (Miami).

In vitro and *in planta* studies identified fungicides with activity against, respectively, *R. lauricola* and laurel wilt. Several chemistries impacted the fungus *in vitro*, but only demethylation inhibitors and thiabendazole provided significant disease control in greenhouse trials. Field investigations are underway to investigate how and whether effective and long-lasting concentrations of triazoles and thiabendazole might be achieved via macroinfusion or other application measures. Although it will be difficult to control this disease by managing *X. glabratus*, insecticides and repellents might ultimately provide useful tools in the holistic management of this disease. Laurel wilt will be a difficult management problem. Fungicides, tolerant germplasm, sanitation and various chemicals for managing the insect vector of the pathogen may ultimately all be useful when combating this important and destructive new disease.

Laurel wilt is an unusual disease. Ambrosia beetle symbionts are usually saprobes, but *R. lauricola* is a virulent, systemic pathogen. In addition, ambrosia beetles typically interact with dead or stressed trees, yet *X. glabratus* is attracted to healthy trees. It is presumed that a visit by a single *X. glabratus* female is sufficient to inoculate a healthy tree and result in systemic disease development (Fraedrich et al. 2008). To date, three other plant pathogens have been reported in *Raffaelea. Raffaelea quercivora* and *R. quercus-mongolicae* cause diseases of *Quercus* spp. in, respectively, Japan (Murata et al. 2007) and Korea (Kim et al. 2009). They are symbionts of their ambrosia beetle vectors, respectively *Platypus quercivorus* and *P. koryoensis*, but unlike *R. lauricola* are not systemic pathogens. Rather, mortality in their host trees is associated with mass attack by the beetle vectors and narrow zones of necrosis that the pathogens cause around the beetles' natal galleries; numerous galleries are needed to girdle stems and kill trees (Takahashi et al. 2010).

Recently, *R. canadensis* was reported to cause symptoms of "laurel wilt" on avocado in California (Eskalen and McDonald 2011). Although ambrosia beetle activity was observed on dead branches on the tree from which the fungus was recovered, an ambrosia beetle association was not demonstrated for the fungus. Since *R. canadensis* caused vascular discoloration, but not mortality, in inoculated

trees, and because the fungus was recovered from only a single tree, it is not clear whether this fungus is a significant pathogen of avocado.

Raffaelea lauricola is unique among the above species in that a single infection by it is sufficient to kill avocado and other host tree species (Fraedrich et al. 2008, Ploetz et al. 2010, 2011c). Our results indicate that laurel wilt rapidly and dramatically decreases the host's ability to transport water, and that these reductions are closely associated with the development of wilting and foliar necrosis symptoms of this disease.

Acknowldgements

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

Cultivars[x]	Genome[y]	Mean external disease severity[z]				
		2008	2009	2010	2008-2010	Genome
'Ettinger'	GxM	n/t	2.8 efg	n/t	-	2.8 b
'Hass'	GxM	3.8 bc	2.7 efg	2.7 fghi	3.1 bcdef	
'Pinkerton'	GxM	n/t	n/t	3.3 defghi	3.3 bcdef	
'Winter Mexican'	GxM	n/t	1.8 g	2.3 hi	2.1 f	
'Bacon'	G	n/t	2.2 fg	2.0 i	2.1fe	2.5 b
*'Marcus Pumpkin'	G	n/t	n/t	2.3 hi	-	
* 'Reed'	G	n/t	3.5 cdefg	n/t	-	
* 'Brogdon'	GxMxWI	4.0 bc	4.1abcdef	4.1 bcdefg	4.1 abcdef	-
'Oro Negro'	MxWI	n/t	n/t	2.5 ghi	-	-
* 'Beta'	GxWI	n/t	3.5 cdefg	4.5 abcde	4.0 abcdef	3.9 ab
* 'Choquette'	GxWI	3.4 c	3.6 cdefg	2.6 fghi	3.2 bcdef	
* 'Hall'	GxWI	3.2 c	4.9 abcd	n/t	4.1 abcdef	
* 'Lula'	GxWI	5.7 a	3.1 defg	5.0 abcd	4.6 abcd	
* 'Miguel'	GxWI	6.0 a	3.7 bcdefg	n/t	4.9 abc	
* 'Monroe'	GxWI	5.2 ab	2.9 defg	3.3 defghi	3.8 abcdef	
* 'Tonnage'	GxWI	n/t	3.5 cdefg	3.0 efghi	3.3 bcdef	
'Bernecker'	WI	5.2 ab	4.2 abcde	3.8 efgh	4.4 abcde	4.8 a
'Catalina'	WI	4.8 ab	5.4 abc	3.5 cdefghi	4.6 abcd	
'Day'	WI	n/t	4.3 abcde	n/t	-	
* 'Donnie'	WI	6.2 a	4.5 abcde	5.4 ab	5.4 ab	
* 'Hardee'	WI	n/t	n/t	4.3 abcdef	4.3 abcdef	
* 'Pollack'	WI	n/t	3.7 bcdefg	n/t	-	
* 'Russell'	WI	n/t	5.6 ab	5.1 abc	5.4 ab	
* 'Simmonds'	WI	6.3 a	5.8 a	5.8 a	6.0 a	
'Trapp'	WI	n/t	3.3 defg	n/t	-	
* 'Waldin'	WI	n/t	4.3 abcde	n/t	-	

[w] Plants were artificially inoculated with mycelium (2008) or conidia (2009) of *Raffaelea lauricola* in field experiments at University of Florida's Plant Science Research and Education Unit in Citra. Data are mean disease severities for three (2008) or two (2009) experiments on a given cultivar. Experiments in 2008 were originally rated on a 1-5 scale, and those in 2009 on a 1-10 scale (see text). To facilitate direct comparisons with data from 2009, the 2008 scores were doubled.

[x] Plants were purchased from a commercial nursery. Clonal scions of cultivars were grafted on seedling rootstocks. Those that are recommended for use in Florida are marked with an asterisk (Crane et al. 2007).

[y] 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 GxWI hybrid, G x Mexican (M) (*P. americana* var. *drymifolia*) hybrid, or a complex GxMxWI hybrid (Chen et al. 2009; Schnell et al. 2003). Since GxWI and WI cultivars predominate in commercial production in Florida, they were tested most frequently in these studies.

[z] n/t=not tested. Mean disease responses are separated with DMRT, $P<0.05$.

Table 2. Fungicides evaluated for in vitro activity against *Raffaelea lauricola* and efficacy against laurel wilt on avocado[a]

Group name	Chemical group	FRAC code	Active ingredient	Trade name[b]	Experiments[c]		
					ED_{50}	Regression line, r^2, *P*>F	Efficacy
chloronitrile	chloronitrile	M5	chlorothalonil	Daconil Ultrex	0.80	y=-24.2x+47.6, 0.92, <0.0001	n.t.
demethylation inhibitor (DMI)	piperazine	3	triforine	Funginex	51.6	y=-36.87x+88.1, 0.99, <0.0001	n.t.
DMI	pyrimidine	3	fenarimol	Vintage SC	0.004	y=-0.07x+0.82, 0.66, <0.0001	4
DMI	triazole	3	flutriafol	Topguard	n.t.	n.t.	4
DMI	triazole	3	myclobutanil	Eagle 20EW	0.10	y=-42.5x+8.2, 0.97, <0.0001	4
DMI	triazole	3	propiconazole	Alamo	0.005	y=-44.1x-53.0, 0.89, < 0.0001	1
DMI	triazole	3	propiconazole	Prophesy 0.72G	n.t.	n.t.	1
DMI	triazole	3	propiconazole	Tilt	n.t.	n.t.	2,3,4
DMI	triazole	3	prothioconazole	Proline 480SC	0.038	y=-16.6x-26.3, 0.94, <0.0001	1
DMI	triazole	3	triadimenol	Baytan 30	0.039	y=-30.7x+6.7, 0.91, <0.0001	1,2
DMI	triazole	3	triadimefon	Bayleton FLO	0.053	y=-42.3x-3.8, 0.90, <0.0001	n.t.
DMI	triazole	3	triticonazole	BAS 595	0.006	y=-31.6x-21.4, 0.98, <0.0001	n.t.
dithiocarbamate	dithiocarbamate	M3	mancozeb	Manzate 200F	2.5	y=-27.9x+61.1, 0.98, 0.0001	n.t.
heteroaromatic	1,2,4-thiadiazole	14	etridiazole	Terramaster 4EC	35.6	y=-32.7x+101, 0.99, <0.0001	n.t.
methyl benzimidazole carbamate	benzimidazole	1	thiabendazole	Arbotect 20S	0.263	y=-22.2x+37.1, 0.88, <0.0001	1,3
phenylamide	acylalanine	4	metalaxyl	Ridomil 2EC	0.65	y=-57.3x-39.3, 0.97, <0.0001	n.t.
phosphonate		33	phosphorus acid salt	Agri-Fos	n.t	n.t.	1
quinone inside inhibitor (Qil)	2,6-dinitro-aniline	29	fluazinam	Omega 500F	0.0004	y=-14.1x+2.4, 0.58, 0.0041	1
quinone outside inhibitor (Qol)	methoxy-acrylate	11	azoxystrobin	Heritage	0.005	y=-19.7x+5.1, 0.90, <0.0001	1,3
Qol	methoxy-carbamate	11	pyraclostrobin	Insignia WG	0.003	y=-9.0x+26.9, 0.35, 0.0152	3
Qol	dihyrdo-dioxazine	11	fluoxastrobin	Disarm	0.0009	y=-7.9x+25.1, 0.52, 0.0079	n.t.
Qol	oximino acetate	11	trifloxystrobin	Compass 50WG	0.26	y=-14.9x+41.4, 0.76, <0.0001	n.t.
succinate dehydrogenase inhibitor (SDHI)	pyridine-carboxamide	7	boscalid	Emerald 70 WG	93.7	y=-21.4x+92.2, 0.98, <0.0001	n.t.
SDHI	phenyl-benzamide	7	flutolanil	Prostar 70WP	>100	y=-9.0x+82.3, 0.63, 0.0020	n.t.

[a] Group names, chemical groups, and FRAC mode of action/resistance codes are found in Fungicide Resistance Action Committee (2010).

[b] Trade names, formulations and sources of tested products: Agri-fos: Agrichem, Liquid Fertilizer Pty. Ltd., Loganholme, Australia; Alamo, Arbotect, Daconil, Heritage, Ridomil and Tilt: Syngenta Crop Protection, Greensboro, NC; BAS 595, Emerald, Funginex, Insignia and Stature: BASF Corporation, Research Triangle Park, NC; Baytan, Bayleton, Compass, Proline, and Prostar: Bayer Environmental Science, Montvale, NJ; Disarm: Arysta LifeScience North America Corporation, Cary, NC ; Eagle: Dow AgroSciences LLC, Indianapolis, IN; Manzate: E. I. du Pont de Nemours and Company, Wilmington, DE; Omega: ISK Biosciences Corporation, Mentor, OH; Prophesy: The Andersons, Maumee, OH; Terramaster: Uniroyal Chemical Company, Inc., a subsidiary of Compton Corporation, Middelbury, CT; Topguard: Cheminova, Inc., RTP, NC; Vintage SC: Gowan Company, Yuma, AZ.

[c] Fungicides were tested in vitro at 0.001, 0.01, 0.1, 1, and 100 □g of the active ingredient (a.i.) ml^{-1} in malt extract agar (MEA). Linear regressions (mean radial growth rate of *R. lauricola* at a given a.i. concentration/mean growth rate on nonamended MEA plotted vs $\log_{10}$ fungicide concentration) were used to compute ED_{50}s, the fungicide concentrations in □g ml^{-1} that were needed to inhibit growth by 50%; >100 indicates that a fungicide did not reduce growth by at least 50% at 100 μg ml^{-1}. Efficacy indicates the experiment(s) in which a given compound was (were) tested; n.t. = not tested.

Table 3. Greenhouse fungicide efficacy against laurel wilt[z]

Treatment[y]	Experiment 1			Experiment 2			Experiment 3		
	Rate[x]	Mean disease[v]		Rate[x]	Mean disease[v]		Rate[x]	Mean disease[v]	
		External	Internal		External	Internal		External	Internal
Nontreated, mock inoculated	-	1.0e	1.0d	-	1.0c	1.0c	-	1.0d	1.0f
Nontreated, inoculated	-	7.2ab	7.8ab	-	5.6a	7.4ab	-	5.7ab	6.3ab
propiconazole soil drench	1.14	1.0e	2.0d	0.04	6.0a	10.0a	0.04	3.0bcd	6.0ab
	-	-	-	0.21	1.0c	3.6b	0.12	1.0d	1.0f
	-	-	-	0.43	1.0c	1.8c	0.4	1.0d	1.2f
	-	-	-	0.85	1.0c	1.0c	0.8	1.0d	1.0f
	-	-	-	1.29	1.0c	1.0c	-	-	-
propiconazole topical bark	0.12	1.0e	1.8d	0.004	4.0ab	7.0ab	0.004	1.6cd	3.2bcdef
	-	-	-	0.02	1.0c	1.6c	0.012	1.8cd	2.6cdef
	-	-	-	0.13	1.0c	1.4c	0.04	1.0d	1.6ef
	-	-	-	-	-	-	0.4	1.0d	1.4f
triadimenol soil drench	0.04	1.0e	2.2d	0.04	1.2c	3.6b	-	-	-
	-	-	-	0.21	1.0c	1.0c	-	-	-
	-	-	-	0.43	1.0c	1.4c	-	-	-
	-	-	-	0.85	1.0c	1.2c	-	-	-
	-	-	-	1.29	1.0c	1.0c	-	-	-
triadimenol topical bark	0.004	1.0e	1.0d	0.004	3.2bc	8.2ab	-	-	-
	-	-	-	0.02	1.0c	1.0c	-	-	-
	-	-	-	0.13	1.0c	1.0c	-	-	-
thiabendazole soil drench	2.5	1.8de	2.8d	-	-	-	0.012	3.4abcd	4.8abcde
	-	-	-	-	-	-	0.4	2.2cd	3.4bcdef
	-	-	-	-	-	-	1.2	1.6cd	2.4def
	-	-	-	-	-	-	4.0	1.2cd	1.6f
thiabendazole topical bark	0.25	6.8ab	8.8ab	-	-	-	0.04	6.4a	6.8a
	-	-	-	-	-	-	0.12	3.4abcd	5.0abcd
	-	-	-	-	-	-	0.4	2.0cd	3.8abcdef
	-	-	-	-	-	-	1.2	1.0d	4.8abcde
azoxystrobin soil drench	0.012	8.4a	10.0a	-	-	-	-	-	-
	-	-	-	-	-	-	0.04	2.8bcd	3.8abcdef

[z]Grafted 'Simmonds' plants in 12 L pots were used. Stem diameters for plants averaged 2.5 cm. Treatments were replicated five times in randomized, complete block designs. Fungicide treatments were imposed 3 weeks before all but the mock inoculated plants were inoculated with $1x10^5$ conidia of *R. lauricola* R14.

[y]Fungicides for the different active ingredients (a.i.s) were: propiconazole: experiment 1, Alamo, and experiments 2 and 3, Tilt; triadimenol: experiments 1 and 2, Baytan 30; thiabendazole: experiments 1 and 3, Arbotect 20S; and azoxystrobin: experiments 1 and 3, Heritage. Manufacturers and a.i. concentrations for each product are listed in Table 3. For each treated plant, applications were made as soil drenches in 1 L of water, or as topical applications to bark with Solo misters in 100 ml aqueous 2% solutions of Pentrabark.

[x] Fungicide rates are g a.i. per cm stem diameter of a treated plant. Mean stem diameters were used to calculate rates.

[v] Five weeks after inoculation, disease severity was estimated visually using a 1-10 scale (1 = no symptoms/healthy and 10 = completely symptomatic). Externally, the extent of canopy wilting and necrosis was evaluated, and internally the extent of sapwood/xylem discoloration caused by laurel wilt was estimated after bark was removed from the stem surface with a knife. Severities are means of five replications, and are separated with Duncan's Multiple Range Test at 0.05.