Executive Summary

Laurel wilt kills American members of the Lauraceae plant family, including avocado (*Persea americana*), an important commercial fruit crop. The disease threatens commercial production in the US and other countries, and currently impacts the avocado industry in Florida. As laurel wilt spreads, the National Germplasm Repository for avocado in Miami (USDA-ARS) and commercial and residential production in other states (e.g. California and Hawaii), US protectorates (Puerto Rico), and other countries are at risk. In the US, value-added production of avocado of more than \$1.3 billion yr^{-1} is threatened.

Laurel wilt is caused by *Raffaelea lauricola*, a nutritional symbiont of an Asian ambrosia beetle, *Xyleborus glabratus*. Laurel wilt was first recognized around Savannah, Georgia in 2003, and has since devastated native populations of redbay (*P. borbonia*) and other native species in the family in the southeastern US. The first avocado tree was killed by laurel wilt in Jacksonville, Florida (2006), and soon after a southward swath of host trees began to die down the eastern flank of the state. In February 2011, the disease was confirmed adjacent to the Everglades on swamp bay (*P. palustris*), and by November of that year had spread to Florida's primary commercial avocado production area (CAPA) in southeastern Miami-Dade County. Within 2 years, the disease had spread throughout the CAPA. Where insufficient or delayed implementation of control measures were used, the disease spread rapidly resulting in lost commercial viability and orchard abandonment. Avocado production continues in areas of Florida to which laurel wilt has not spread and where the disease has been effectively managed. Excluding laurel wilt from healthy avocado orchards and managing the disease in affected orchards is a major, ongoing challenge.

Laurel wilt has spread along the eastern seaboard of the US due to a mobile insect vector, *X. glabratus*, the movement of wood infested with the insect and pathogen, and the presence of native and non-native plants that are susceptible to the disease and in which *X. glabratus* reproduces. Rapid spread has occurred where there are (were) high population densities of redbay and swamp bay. In avocado, the ambrosia beetles that disseminate *R. lauricola* are unclear. In addition, the pathogen likely moves via avocado root grafts, but dissemination via pruning equipment, fruit, seed or scion material is unlikely.

Diverse disease management strategies have been examined for avocado, including host resistance and the use of fungicides and insecticides. To date, no highly efficacious and cost-effective measure has been identified. In the absence of such a measure, holistic considerations of host tolerance and chemical mitigation will be needed. In addition, cultural measures, notably the prompt identification and removal of infected trees (sanitation) is needed to ensure that pathogen movement to adjacent trees via root grafts, as well as the reproduction of ambrosia beetle vectors, does not occur or is minimized.

Unexpected insights have emerged with nearly every new research finding on this disease. Laurel wilt is unique in that symbionts of ambrosia beetles are rarely plant pathogens and were not known previously to be systemic (one inoculation with *R. lauricola* is sufficient to colonize and kill an entire tree). Lateral transfer of the pathogen to at least nine other species of ambrosia beetle has occurred since *X. glabratus* was introduced to the US, some of which have transmitted the pathogen to redbay and avocado experimentally. Surprisingly, *X. glabratus* is rarely trapped in laurel wilt-affected commercial orchards and reared from laurel wilt-affected bolts from the CAPA. Root-graft transmission of the pathogen, difficult detection and inconsistent distributions of the fungus in affected trees, poor resistance to the disease in avocado, and the expense of chemical and cultural measures have complicated management efforts. Improved control measures will rely on better understandings of the host x pathogen interaction, as well as the epidemiology of laurel wilt in avocado orchards and surrounding areas. The identities and dynamics of different vectors in the disease cycle, and ways in which pesticide efficacy, longevity and distribution could be increased in treated trees, are also needed.

American members of the Lauraceae are usually more susceptible to the disease than are those from the beetle’s Asian home range. Limited information is available on the extent to which lauraceous and non-lauraceous taxa serve as hosts and reservoirs for *R. lauricola*, *X. glabratus* and other potential ambrosia beetle vectors. Features of laurel-wilt tolerant, Asian taxa in the Lauraceae (e.g. camphortree, *Cinnamomum camphora*) may provide clues on attributes that would enhance resistance in different avocado genotypes.

Recommended Next Steps:

Good progress has been made in understanding this disease since 2004. Nonetheless, continued work on the most pressing issues is still required, as it is still unclear what actions would be used cost-effectively in commercial avocado-production areas.

1. Research on the efficacy and cost-effectiveness of chemical controls measures (fungicides, and insecticides, attractants and repellents of ambrosia beetle vectors) must continue as they may ultimately provide important components of a multifaceted management scheme.
2. Ongoing work to identify disease tolerance in avocado should continue. Thus far, it appears that insufficient tolerance exists among the cultivars that are currently grown in Florida, and that new cultivars and genotypes may be needed if resistance is to play a significant role in addressing this disease. Currently, manipulation of the response of susceptible West Indian cultivars is being investigated with different clonal rootstocks, as the selection and development of resistant and commercially acceptable cultivars would be a long process.
3. Data are needed on the impact of ambrosia beetles other than *X. glabratus* in the movement of *R. lauricola* to healthy avocado and other lauraceous taxa, and the extent to which they spread the disease.
4. State, federal and international regulations on the movement of firewood and untreated yard and forest waste are needed, as the long-distance transport of the same has resulted in significant jumps in the distribution of this disease during its brief history in the southeastern US and will likely result in the continued spread of this disease. Regulations recently enacted or proposed in Florida (see VI. Permit and Regulatory Issues) provide useful models for what could be considered in other states.
5. State and federal efforts to educate stakeholders on the disease and its mitigation should continue.
6. Duplication of important *Avocado sunblotch viroid* (ASBVd)-free accessions in the USDA-ARS repository for avocado germplasm and their transfer and establishment at the USDA-ARS facility in Hilo, HI should continue.

Recovery Plan for Laurel Wilt of Avocado, caused by *Raffaelea lauricola*

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension, and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Citation: Ploetz, R.C., Hughes, M.A., Kendra, P.E., Fraedrich, S.W., Carrillo, D., Stelinski, L.L., Hulcr, J., Mayfield, A.E. III, Dreaden, T.L., Crane, J.H., Evans, E.A., Schaffer, B.A., and Rollins, J. 2016. Recovery Plan for Laurel Wilt of Avocado, caused by *Raffaelea lauricola.* National Plant Disease Recovery System. Homeland Security Presidential Directive Number 9 (HSPD-9). xx pp. http://www.ars.usda.gov/Research/docs......

I. Introduction

Laurel wilt affects members of the Lauraceae plant family (Laurales, Magnoliid complex), and is caused by a fungal symbiont, *Raffaelea lauricola* (Ophiostomatales), of an Asian ambrosia beetle, *Xyleborus glabratus* (Curculionidae: Scolytinae) (Fraedrich et al., 2008). Starting in its probable epicenter of Port Wentworth, Georgia, the disease had been confirmed by August 2016 in nine states as far west as Hardin County, Texas (30°N and 95°W), as far east as Onslow County, North Carolina (34°N and 78.5°W), as far north as Sampson County, North Carolina (35°N and 79°W), and as far south as Miami-Dade County, Florida (25.5°N and 80.5°W) (Barton et al. 2016). (Fig. 1). Although virtually all of these outbreaks have occurred on native host trees (Hughes et al., 2015b), commercial production of a tropical American crop in the family, avocado (*Persea americana*), was affected in Florida beginning in 2012 (Ploetz et al., 2013).

Harrington et al. (2011) recovered *R. lauricola* from specimens of *X. glabratus* from Japan and Taiwan, corroborating the assumption that the pathogen arrived in the US with *X. glabratus*. Since there are no reports of laurel wilt prior to 2003, it is probable that: i) the beetle carried the pathogen when it was first detected in Port Wentworth in 2002 (Haack, 2006; Rabaglia et al., 2006); ii) that introduction established the beetle and pathogen in the US (Harrington et al., 2011); and iii) American suscepts in the Lauraceae are all new encounter, so-called naïve hosts (Ploetz et al., 2013). A single, asexually reproducing clone of *R. lauricola* is responsible for the laurel wilt epidemic in the US (Hughes, 2013).

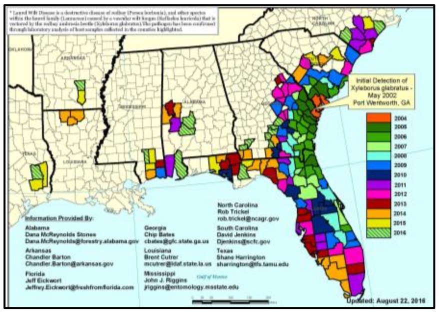


Figure 1. Laurel wilt distribution in southeastern USA

Although some species, such as *Xylosandrus compactus*, cause primary damage (Ngoan et al., 1976; Ranger et al., 2010), ambrosia beetles usually colonize and reproduce in stressed or dead trees. This has led to a general assumption that most ambrosia beetle species do not interact with healthy, non-stressed trees, and that the interaction between *X. glabratus* and trees that are susceptible to laurel wilt is unusual, since healthy trees are attacked to initiate the disease cycle (Hulcr and Dunn, 2011; Kuhnholz et al., 2002). However, how *X. glabratus* colonizes trees is poorly understood. Fraedrich et al. (2008) indicated that *X. glabratus* may not colonize healthy host trees, but that its initial interaction with these trees is sufficient to infect them with *R. lauricola*. A primary reason this beetle is known to interact with healthy trees is that it carries a lethal, systemic pathogen. If other beetle species interacted with healthy trees their activities might not be recognized unless they caused conspicuous damage or carried a lethal pathogen (Ploetz et al., 2013).

Critical gaps exist in what is known about the development and epidemiology of laurel wilt on avocado. The interaction of susceptible host tree species and *R. lauricola* is incompletely understood, as is the nature of tolerance to the disease (Ploetz et al., 2015). Information is needed on pathogen and host attributes that result in compatible and incompatible disease responses, as it could enable the selection of resistant genotypes and may assist the development of better disease diagnostics. Considering the large number of species in, and the wide geographic distribution of, the Lauraceae, more data are needed on the reactions of different species in the family to the pathogen as they could help improve management strategies, predict and explain the disease's spread, and plan research on the host x pathogen interaction. Much remains to be learned about the identity and biology of vector(s) of this pathogen. *Xyleborus glabratus* is rare in the CAPA. Since *R. lauricola* is carried by several other ambrosia beetle species in the CAPA, which have experimentally transmitted the pathogen to avocado and swamp bay (*Persea palustris*), the role of species other than *X. glabratus* should be considered (Carrillo et al., 2014; Ploetz et al., 2013; 2016a). Little is known about the interaction of *R. lauricola* and other beetles that carry the pathogen, and virtually nothing is known about the management and attraction of the other species to avocado.

II. Disease Cycle and Symptom Development

Although details are known about the *X. glabratus* interaction with several host trees in the US (Hughes et al., 2015b; Kendra et al., 2014a), very little is known about the identity and life cycles of the vectors in the avocado system (see III. Spread). This section deals primarily with the host x pathogen interaction.

Inoculation with as few as 100 conidia of *R. lauricola* can kill avocado and swamp bay (Hughes et al., 2015a). Xylem function (the ability to conduct water) is impaired as soon as 3 days after inoculation, before the development of external or internal symptoms of the disease are apparent (Fig. 2) (Inch et al., 2012). Reductions in xylem function that develop in these plants are correlated with increased tylose formation in xylem lumena and the eventual development of symptoms (Figs. 2 and 3) (Inch et al., 2012; Inch and Ploetz, 2012). Tree mortality is associated with functionalities of less than 10%.

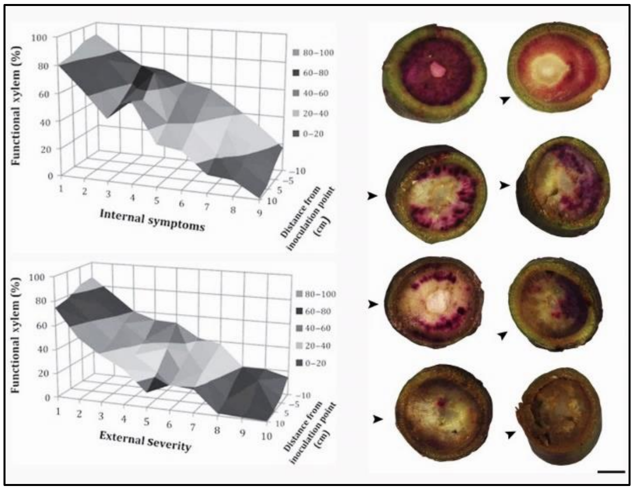


Figure 2. 'Simmonds' avocado trees were inoculated with *Raffaelea lauricola* and examined for: i) the development of internal (sapwood discoloration) and external (foliar wilting and necrosis) symptoms of laurel wilt on a 1-10 scale, wherein 1=healthy, no symptoms, and 10=dead, totally symptomatic (response surface graphs on the left), and ii) the ability of xylem to conduct water (functional xylem on the left and in the stem sections on the right), as determined with an acid fuchsin assay. In the stem sections, burgundy to pink coloration indicates functional xylem, which was quantified in scanned, digital images. From left to right and top to bottom, xylem in cross sections are: 98% functional (water control, internal symptoms (is) = 1; 86% (3 days after inoculation (dai), is = 1; 76% (7 dai, is = 2); 71% (14 dai, is = 3); 32% (21 dai, is = 5); 30% (21 dai, is = 6; 5% (42 dai, is = 9; and 1% (42 dai, is = 9). Scale bar = 0.5 cm.

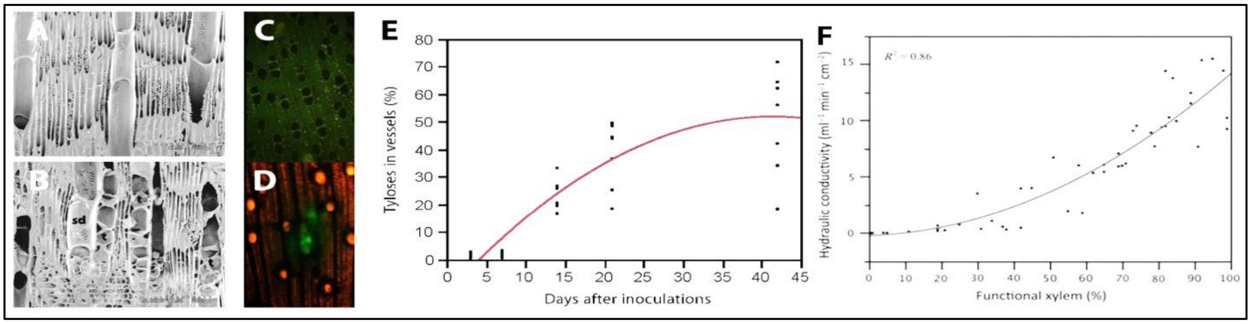


Figure 3. *Raffaelea lauricola* induces tylose formation in infected avocado trees, but is scarcely evident, microscopically. In scanning electron micrographs, tyloses are (A) absent in vessel lumena in noninoculated trees, but are (B) prevalent 21 days after inoculation. Ten days after inoculation, a GFP-marked strain of the pathogen is: (C) not visible, until (D) 5 days after a bioenrichment step. The percentage of vessel cross-sections that are occluded with tyloses (E) increases soon after inoculation ($R^2 = 0.78$; $P < 0.0001$; $y = -0.69 + 1.79x - 0.04\,(x-17.4)^2$), and xylem dysfunction that is associated with tylose presence (F) is related to reduced hydraulic conductivity ($R^2 = 0.86$; $P < 0.001$; $y = -4.25 + 0.16x + 0.001x^2$).

Tylose induction in susceptible cultivars is poorly understood. Although a maximum of 0.4% of the lumena were colonized by a green fluorescent protein (gfp)-labelled strain of the pathogen in microscopic cross sections, 30 days after inoculation (Fig. 4A) (Campbell et al., 2016), in another study about 40% of the lumena were occluded by tyloses 21 days after inoculation (Fig. 3E) (Inch et al., 2012). Mobility of the pathogen (or its metabolites) in the xylem seems to be related to susceptibility, since sap flow rates were significantly higher in a susceptible versus less susceptible cultivars, before inoculation (Ploetz et al., 2015); however, sap flow plummeted in the susceptible cultivar soon after inoculation. In the analogous Dutch elm disease pathosystem, relatively high sap flow rates were predictors of susceptibility (Solla et al., 2005; Venturas et al., 2014).

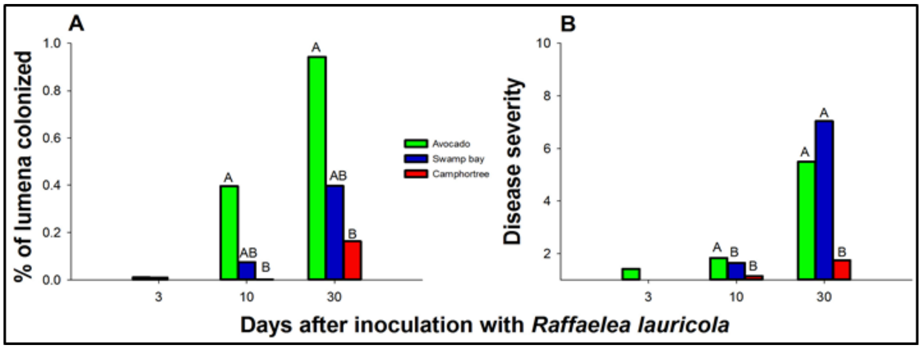


Figure 4. **Avocado**, **swamp bay** and **camphortree** respond differently to artificial inoculation with *Raffaelea lauricola.* After 30 days, (A) colonization of lumena cross-sections by a gfp-marked strain of the pathogen varied by host, and (B) more disease developed on **swamp bay** and **avocado**, than on **camphortree.**

Colonization of host trees in the Lauraceae by *R. lauricola* was investigated by Campbell et al. (2016). Moderate to severe disease developed on trees of avocado and swamp bay, whereas little to no disease developed on camphortree (*Cinnamomum camphora*) (Fig. 4B). Susceptibility and the extent to which these hosts were colonized were related, in that a greater microscopic presence of *R. lauricola* in xylem lumena and greater colonization of sapwood were observed in swampbay and avocado compared to that in camphortree (Figs. 4A and 5).

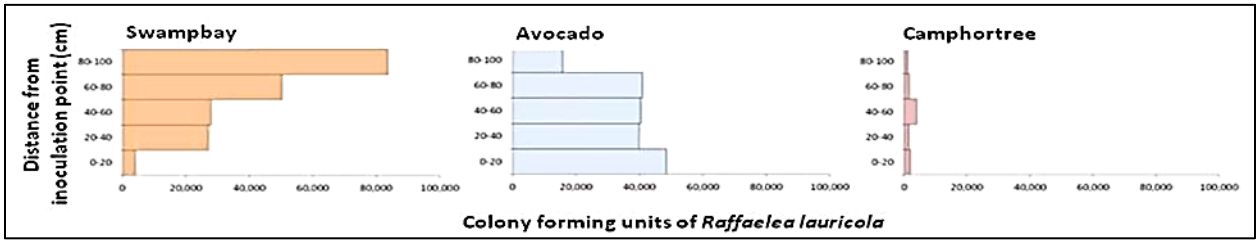


Figure 5. Thirty days after inoculation, colonization of swampbay, avocado and camphortree by *Raffaelea lauricola*, quantitated as colony forming units of the pathogen per gram of sapwood, varied by species and distance from the inoculation point.

Some of the symptoms of laurel wilt on avocado resemble those caused by other diseases or abiotic factors. Lightning, over-production of fruit and frost damage can kill all or portions of tree canopies, as can different diseases, in particular Phytophthora root rot, caused by *P. cinnamomi*, and Verticillium wilt, caused by *V. dahliae*. Vascular discoloration similar to that caused by laurel wilt also develops in trees affected by Verticillium wilt.

The first external, foliar symptoms of laurel wilt on avocado are wilting of terminal leaves that change from an oily green to brown color soon after wilting (Fig. 6A). Symptoms typically develop rapidly in affected portions of the tree (Fig. 6B), but production of healthy branches beneath affected regions in the tree (Fig. 6C) or the unilateral development of symptoms in which only a branch or a portion of a tree are affected also occurs (Fig. 6B). Unlike laurel wilt-affected redbay (*Persea borbonia*), which retains dead leaves for a year or longer, avocado defoliates within 2-9 months of symptom initiation (Fig. 6C). Internally, affected avocado sapwood is discolored reddish brown to bluish grey (Fig. 6D).

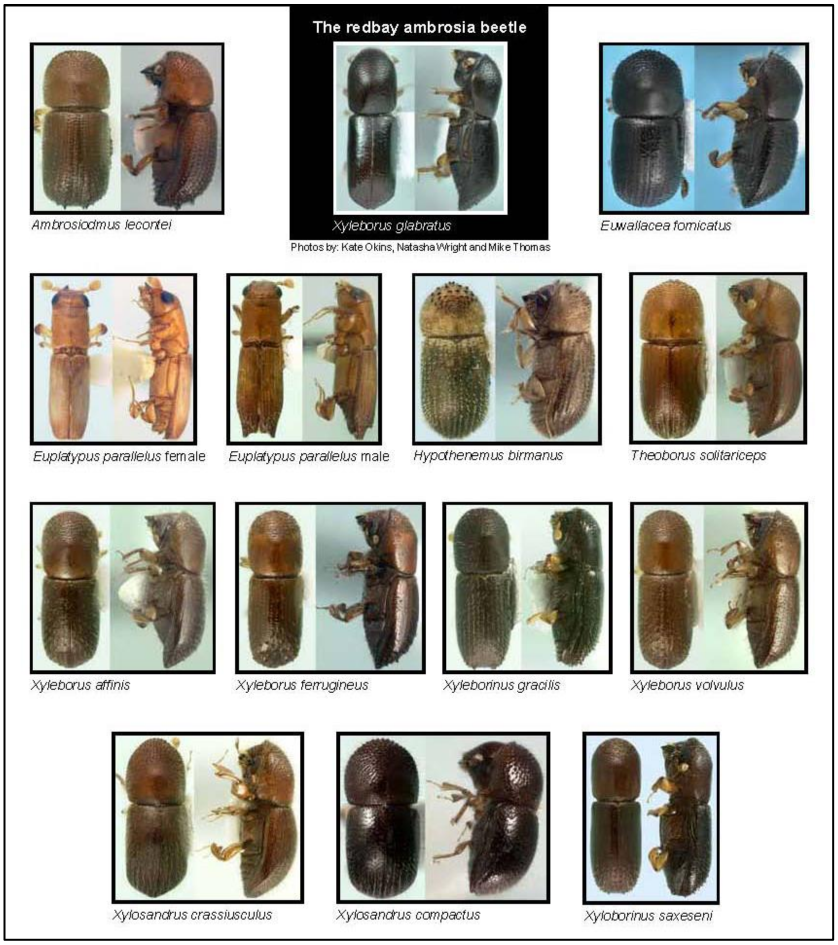
In artificial inoculation studies, moderate internal symptoms develop on avocado before external symptoms are evident (Ploetz et al., 2012b). On a 1-10 scale, where 1 = no symptoms and 10 = dead or completely symptomatic, internal severities can reach 5 before external severities are evident (Fig. 2). The threshold between internal and external symptoms has important implications for disease management since it is doubtful that systemic fungicides would be very effective if they were applied after external symptoms of the disease began to develop, due to decreased mobility and distribution of fungicides in the nonfunctional xylem in such trees (Figs. 2 and 3).

III. Spread

Lateral movement of fungal symbionts from one species to another is known in both bark beetles (Six, 2003) and ambrosia beetles (Batra, 1966; Carrillo et al., 2014; Gebhardt et al. 2004, Kostovcik et al. 2015, Ploetz et al. 2016a). To date, 17 ambrosia beetle species have been detected on avocado (Carrillo et al., 2012; 2014; unpublished; Kendra et al. 2011b) (Fig. 7). In the US, *R. lauricola* has been isolated from nine species of ambrosia beetles other than *X. glabratus* (Carrillo et al., 2014; Harrington and Fraedrich, 2010; Ploetz et al., 2012; 2016a). All of the other species occurred in the US prior to the introduction of *X. glabratus* and, presumably, obtained the fungus in laurel wilt-affected trees after that introduction (Ploetz et al., 2013).

Figure 6. Symptoms of laurel wilt on avocado include: (A) oily green foliage that necroses to a brown color, (B) sectoral development of symptoms in which only a portion of the tree is affected, (C) defoliation in portions of the tree that developed symptoms first, and (D) and (E) discoloration of sapwood. (A) to (C) are 'Simmonds' trees that were artificially inoculated with *Raffaelea lauricola*, and (D) and (E) are from branches of fruit-bearing trees in the field (photos: R.C. Ploetz).

More propagules of *R. lauricola* have been detected in *X. glabratus* than in any of the other species (Carrillo et al., 2014; Harrington et al. 2010; Ploetz et al., 2016a). However*, X. glabratus* appears to be far more prevalent and important on native trees from the southeastern US than on avocado (Carrillo et al., 2012; Hanula et al., 2008; Mayfield and Hanula, 2012). In recent surveys in Miami-Dade County, Florida, it was the most prevalent species recovered from laurel wilt-affected swamp bay (47.8% of all identified individuals), but was not trapped in laurel wilt-affected avocado orchards or reared from laurel wilt-affected avocado wood (0 of 79,025 ambrosia beetles that were recovered) (Table 1). And in an earlier survey in Brevard County *X. glabratus* was reared from two of four laurel wilt-affected avocado trees, but at very low numbers (11 of a total of 4,181 individual beetles that were collected) (Carrillo et al., 2012). Thus, despite its prevalence in swamp bay, *X. glabratus* has hardly ever been associated with avocado.



Photos by: Kate Okins, Natasha Wright and Mike Thomas

Figure 7. Seventeen ambrosia beetle species have been recovered from avocado trees in Florida, 13 of which are illustrated here. The primary vector of *Raffaelea lauricola* in native host trees in the southeastern US, *Xyleborus glabratus*, is shown in the middle of the top row. It is rarely found on avocado trees in Florida (Photos: DPI, Gainesville, FL).

The rarity of *X. glabratus* in avocado orchards in South Florida and the presence of *R. lauricola* in other beetle species suggests that other species could be vectors in the avocado pathosystem. *Raffaelea lauricola* has been recovered consistently from *Xyleborus ferrugineus*, *Xyleborus bispinatus* and *Xyleborus volvulus* that were reared from swamp bay and avocado, but was found infrequently in two of the most common species in swamp bay and avocado, *Xylosandrus crassiusculus* and *Xyleborinus saxeseni*, and was present in *Xyleborus affinis* and *Xyleborinus gracilis* from swamp bay but not from avocado (Tables 2 and 3). Among the most common species that were associated with avocado (Table 1), only reared *Xyleborus volvulus* harbored appreciable amounts of the pathogen (Table 3). Overall, *R. lauricola* was recovered less often

Table 1. Recovery of ambrosia beetles from laurel wilt-affected avocado orchards and trees in Miami-Dade County, Florida

	Avocado				**Swamp bay**	
	Trapped in orchards [z]		**Reared from bolts[y]**		**Reared from bolts[y]**	
Species	**n [w]**	**% total**	**n [w]**	**% total**	**n [w]**	**% total**
Xyleborus glabratus	0	0	0	0	25,823	47.8
Xyleborus affinis	13,814	22.0	2,053	12.7	4,411	8.2
Xyleborus bispinatus	1,348	2.1	69	0.4	- [x]	- [x]
Xyleborus ferrugineus	441	0.7	53	0.3	3,456	6.4
Xyleborus volvulus	7,274	11.6	1,856	11.5	9,685	17.9
Xylosandrus crassiusculus	11,761	18.7	8,533	52.7	1,479	2.7
Xyleborinus gracilis	117	0.2	47	0.3	8,097	15.0
Xyleborinus saxeseni	28,067	44.7	3,592	22.2	422	0.8
Totals	62,822		16,203		53,979	
Grand totals	79,025				53,979	

[z] Beetles were trapped in 13 avocado groves that were affected by laurel wilt.

[y] Bolts from 75 laurel wilt-affected avocado trees and 17 swamp bay trees were incubated for recovery of ambrosia beetles as described by Carrillo et al. (2012; 2014).

[w] n = total numbers of a given species that were recovered.

[X] *Xyleborus bispinatus* was distinguished from *Xyleborus ferrugineus* in latter work on avocado, but not during work on swampbay (Atkinson et al., 2013; Carrillo et al., 2012).

Table 2. Recovery of *Raffaelea lauricola* from ambrosia beetles reared from laurel wilt-affected swamp bay trees [z]

Species	n [z]	No. w *R. lauricola*	% of beetles w *R. lauricola* (%)	CFU mean ± SEM [z]	CFU range [z]
Xyleborus glabratus[y]	50	43	86 a	2783.3 ± 281.9 a	0–7800
Xyleborus affinis	41	5	12 c	1 ± 0.6 c	0–20
Xyleborus ferrugineus[y, x]	118	70	59 b	33 ± 7.4 b	0–118
Xyleborus volvulus	39	20	51 b	28.4 ± 10.6 b	0–100
Xyleborinus gracilis	52	26	50 b	100.6 ± 34 b	0–1240
Xyleborinus saxeseni [y]	68	2	3 c	1.5 ± 1 c	0–60
Xylosandrus crassiusculus[y]	39	1	3 c	2.6 ± 2.6 c	0–100
Ambrosiodmus devexulus[v]	25	0	0	0	0
Ambrosiodmus lecontei[v]	41	0	0	0	0
Total	473		35.3		

[z] Recovery of *Raffaelea lauricola* from individuals of the different species was described by Carrillo et al. (2014). n = number of individuals that were assayed, CFU = colony forming unit of *Raffaelea lauricola* on a semi-selective medium, and SEM = standard error of the mean. Means followed by the same letter within columns are not significantly different at $P < 0.05$. The PROC GLIMMIX procedure (SAS v. 9.3 2012) was used to assess differences in the percentage values, and the Steel–Dwass method (SAS v. 9.3 2012) was used for non-parametric paired comparisons of mean CFUs of *R. lauricola* in the different beetle species.

[y] In olfactometer assays, *Xyleborus glabratus* was attracted to *Raffaelea lauricola*, *Xyleborus ferrugineus* did not respond, and *Xylosandrus crassiusculus* and *Xyleborinus saxeseni* were repelled (Hulcr et al., 2011).

[x] *Xyleborus bispinatus* was distinguished from *Xyleborus ferrugineus* in latter work on avocado, but not during work on swampbay (Atkinson et al., 2013).

from beetles trapped in laurel-affected avocado orchards (mean recovery of 1.6%) than from beetles reared from laurel wilt-affected avocado bolts (11.1%) (Table 3).

That *Xyleborinus saxesenii* and *Xylosandrus crassiusculus* rarely carry *R. lauricola* corresponds with previous indications that they are repelled by the fungus. In olfactometer assays, Hulcr et al. (2011) determined that adult females of these species avoided cultures (volatiles) of the pathogen. Interestingly, another beetle that carried the pathogen more frequently, *Xyleborus ferrugineus*, had a net nonresponse (was repelled about as often as it was attracted; 156 vs 132, *P*=0.16), whereas *X. glabratus* was attracted to *R. lauricola* (in 54 of 84 assays, *P*=0.004) (Hulcr et al., 2011). *X. glabratus* responded synergistically

Table 3. Recovery of *Raffaelea lauricola* (*Rl*) from ambrosia beetles associated with laurel wilt-affected avocado trees [z]

	Trapped in laurel wilt-affected orchards					Reared from laurel wilt-affected bolts				
Species	n[z]	No. w *Rl*	% beetles w *Rl*	CFU mean ± SEM z	CFU range[z]	n[z]	No. w *Rl*	% beetles w *Rl*	CFU mean ± SEM [z]	CFU range[z]
Xyleborus glabratus[y]	0 x	n/a	n/a	n/a	n/a	0 x	n/a	n/a	n/a	n/a
Xyleborus affinis	50	0	0	0	0	16	0	0	0	0
Xyleborus bispinatus	14	1	7	0.1±0.1	0 - 2	5	5	100	40.8±16.1	4 - 80
Xyleborus ferrugineus[y]	2	1	50	50±50	0 - 100	2	2	100	5±1	4 - 6
Xyleborus volvulus	97	2	2	0.4±0.3	0 - 20	53	10	19	30.0±21.8	0 - 1140
Xylosandrus crassiusculus[y]	75	0	0	0	0	24	1	4	15±15	0 - 360
Xyleborinus gracilis	3	0	0	0	0	10	0	0	0	0
Xyleborinus saxeseni [y]	172	4	2	2.5±1.6	0 - 200	51	0	0	0	0
Totals/means	413	8	1.6			161		11.1		

[z] Recovery of *Raffaelea lauricola* from individuals of different species, as described by Carrillo et al. (2014). n = number of individuals that were assayed, CFU = colony forming unit of *Raffaelea lauricola* on a semi-selective medium, and SEM = standard error of the mean.

[y] In olfactometer assays, *Xyleborus glabratus* was attracted to *Raffaelea lauricola*, *Xyleborus ferrugineus* did not respond, and *Xylosandrus crassiusculus* and *Xyleborinus saxeseni* were repelled (Hulcr et al., 2011).

[x] *Xyleborus glabratus* has not been recovered during recent surveys in laurel wilt-affected commercial avocado groves in Florida (see Table 2).

to volatiles from their symbiotic fungi and host plant volatiles, compared to volatiles of the fungus or host plant alone (Kuhns et al. 2014). Infection of redbay with *R. lauricola* increased attractiveness of the host to *X. glabratus* (Martini and Stelinski, unpublished), which may enhance the spread of laurel wilt, as has been shown for other plant diseases with insect vectors (Mann et al. 2012). Recently, Martini et al. (2016) demonstrated that pathogen-induced manipulation of vector behavior can be disrupted. This semiochemical-based strategy is being investigated for the management of laurel wilt.

Understanding how different ambrosia beetles respond to *R. lauricola* and other microbes that they encounter in trees (e.g. other ambrosia beetle symbionts and avocado endophytes) could help identify vectors of the pathogen. Whether a beetle avoids, ignores or is attracted to *R. lauricola* may impact whether it is a factor in the epidemiology of this disease on avocado. Similarly, determining whether and how the symbiotic fungus manipulates volatile release from host trees to affect behavior of the vector is significant. How do common species that are repelled by *R. lauricola*, such as *Xyleborinus saxesenii*, avoid the pathogen in trees that are affected by laurel wilt, and what do different beetle species farm in their natal galleries in laurel wilt-affected avocado trees? Do repelled species weed *R. lauricola* out of their gardens or do their symbionts inhibit its growth? Are beetles that are not repelled by the fungus able to cultivate it and utilize it as a food source, and does *R. lauricola* directly or indirectly manipulate the behavior of *X. glabratus* or other *Xyleborus* species interspecifically? The extent to which these are active decisions or passive functions of the vector x microbial symbiont interaction are generally unknown. Kostovik et al. (2015) recently demonstrated that functionally and taxonomically distinct symbioses could be dictated by the type of mycangium that a given species possessed. Thus, the apparent preferential association between *R. lauricola* and *Xyleborus* congeners may reflect a conducive environment in that genus' mandibular mycangia, in addition to an innate attraction to the fungus (Ploetz et al. 2016a).

The identity of vectors of *R. lauricola* in avocado orchards and information on their reproductive potential, seasonality and flight behavior are needed. A current working hypothesis is that ambrosia beetle species other than *X. glabratus* transmit *R. lauricola* to avocado, but very inefficiently. Once the pathogen infects a tree it moves rapidly throughout the xylem and to adjacent trees via interconnecting roots, in a manner similar to Dutch elm disease, caused by *Ophiostoma* spp., and oak wilt, caused by *Ceratocystis fagacearum* (Sinclair and Lyon, 2005). Understanding which beetle species disseminate the pathogen to healthy trees and the circumstances under which these interactions occur will be critical to the development of strategies to keep unaffected avocado groves free of the disease. Also, understanding the flight capacity of vectors will be critical. Initial laboratory investigations suggest that females of different *Xyleborus* species are capable of many kilometers of continuous flight without wind assistance (Stelinski et al., unpublished). Explorations have only begun on the physiological limits of, and the impact of the symbiont-vector interaction on, their flight capacity.

The primary vector of *R. lauricola* in natural areas is *X. glabratus* (Carrillo et al., 2012; Hanula et al., 2008; Mayfield and Hanula, 2012). Most studies on the life history of this beetle have been conducted in large stands of redbay or swamp bay. In redbay stands in north Florida, adults were active throughout the year; they were trapped in two peaks during March - April and October, and in the greatest numbers between 1600 and 1800 h and 35–100 cm above the ground (Brar et al., 2012).

Mayfield et al. (2008) determined that *X. glabratus* bored into, and transmitted *R. lauricola* to, potted avocado trees in no-choice tests. Peña et al. (2013) reported similar results for avocado cultivars that were not screened by Mayfield et al. (2008), and that *X. glabratus* bored into wood (detached bolts) of other New World species in the Lauraceae. In natural areas in central Florida, Kendra et al. (2011a; 2014a) found that volatiles from avocado wood were attractive to dispersing females of *X. glabratus* (Fig. 8). The emissions of four sesquiterpenes, α-copaene, α-cubebene, α-humulene, and calamenene, was positively

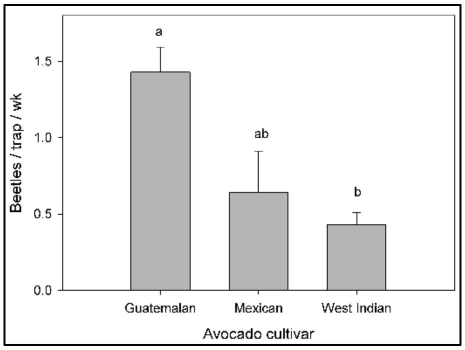


Figure 8. Mean (± SE) captures of *X. glabratus* with freshly-cut bolts of avocado. Cultivars were 'Taylor' (Guatemalan race), 'Duke' (Mexican race), and 'Catalina' (West Indian race).

correlated with field captures. Brar et al. (2013) determined that *X. glabratus* developed at comparable rates in logs of avocado, redbay and swampbay, but that fewer progeny were produced in avocado. Thus, low numbers of *X. glabratus* that have been detected in avocado orchards may be due to avocado being a poor reproductive host.

Carrillo et al. (2014) reported that six and two species other than *X. glabratus* transmitted *R. lauricola* to potted redbay and avocado trees, respectively, and that laurel wilt developed in six and one of the interactions (Fig. 9). Given the rarity of *X. glabratus* in laurel wilt-affected avocado orchards and the ability of other ambrosia beetle species to harbor and transmit the pathogen, it seems probable that other species are capable of spreading *R. lauricola* in commercial settings. Other species could be significant factors in the epidemic on this crop, and might help *R. lauricola* expand its host range (Ploetz et al., 2013), as they usually have broader host ranges than *X. glabratus* (Carrillo et al., 2014; Hulcr and Lou, 2013).

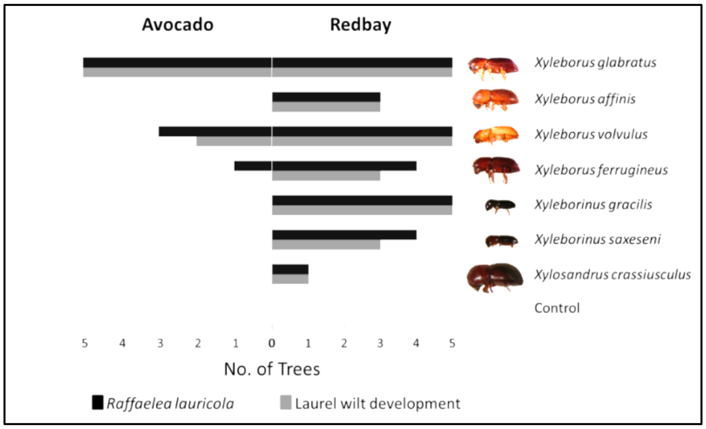


Figure 9. Transmission of *Raffaelea lauricola* to, and the development of laurel wilt in, healthy avocado and redbay trees confronted with seven species of ambrosia beetles under no-choice conditions. Five trees of each host tree were tested with each species of beetle.

Rapid spread of laurel wilt in the southeastern US has been associated with populations of susceptible host species (mainly redbay) and an efficient vector, *X. glabratus*. Spread may have also been exacerbated by the pathogen's promiscuity among different ambrosia beetle species, as noted above. Where redbay was a major component of communities, significant mortality developed within a few years of the onset of an epidemic (Er et al., 2013; Koch and Smith, 2008). Compared to natural environments, a different pattern of spread has been evident in commercial avocado orchards. In the latter settings, high densities of this host are planted in rows in which root grafting occurs among adjacent trees, as shown previously by the movement of herbicides and another avocado pathogen, *Avocado sunblotch viroid* (ASBVd) (Ploetz et al., 2012b; Dann et al., 2013). Typically, laurel wilt develops initially on single trees that decline rapidly. If these trees and their associated stumps are not removed soon after detection, secondary spread occurs both to new foci within an orchard and to adjacent trees (Figs. 10 and 11). New foci are thought to result from the activity of beetle vectors, but spread to adjacent trees in a focus is probably due to root-graft transmission of the pathogen since: i) roots are infected by *R. lauricola* in trees that are naturally affected by laurel wilt; ii) foci expand rapidly in avocado orchards, before vectors could propagate and disseminate *R. lauricola* to surrounding trees; and iii) prompt treatment of adjacent trees with fungicides impedes spread.

Figure 10. Focal development of laurel wilt in an avocado orchard in south Florida. Note spread of disease in the focus on the right in which movement occurred from trees that were first affected, which are defoliated here, to adjacent trees, which retain dead leaves. Also note the movement along rows, in which root grafting is most prevalent. Another newer focus has developed on the left, presumably due to vector dissemination of the pathogen.

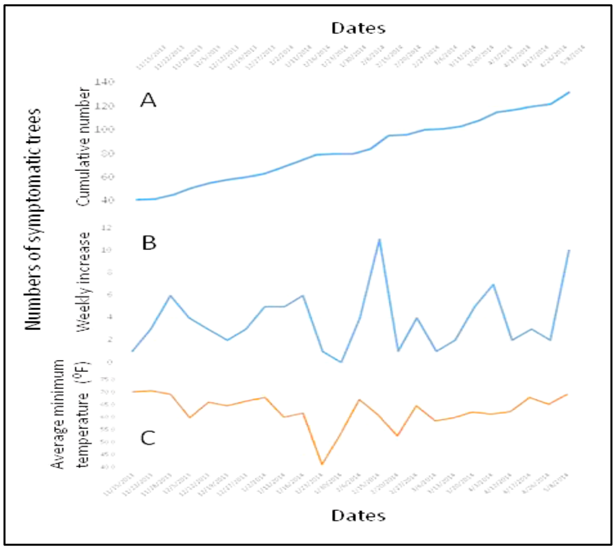


Figure 11. Spread of laurel wilt in an avocado orchard in south Florida in which tree removal and fungicide treatment were not used to manage the disease. Data are weekly summaries for contiguous trees in four disease foci, from November 2013 to May 2014, and are (A) cumulative totals of symptomatic trees and (B) weekly new cases of the disease. The average minimum temperature (ºF) during the same weeks (C), illustrates an association between temperature and disease spread.

Size influences the order in which trees are affected by laurel wilt. Large native host trees are impacted first (Fraedrich et al., 2008; Kendra et al. 2013) (Fig. 12), due to their greater attraction of *X. glabratus*, due to visual cues (Mayfield and Brownie, 2013), and higher content of α-copaene (Niogret et al. 2013). Small trees are less apt to be attacked and may escape infection.

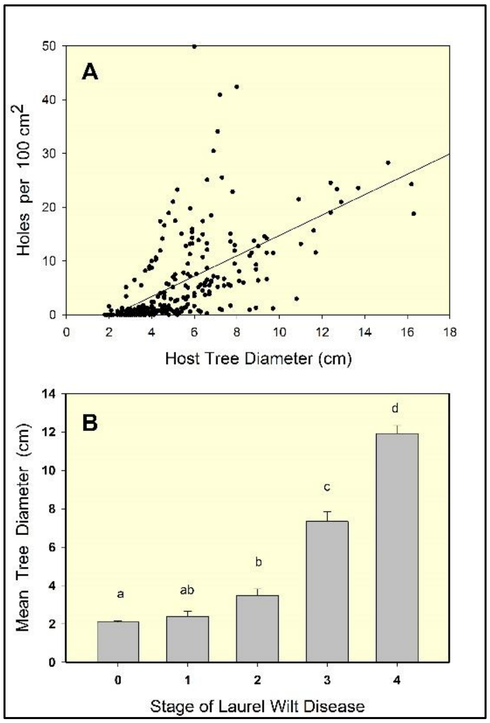


Figure 12. Relationships among host tree diameter, site of *X. glabratus* attack, and stage of laurel wilt development: (A) number of beetle entrance holes (per 100 cm^2) versus the trunk/branch diameter of host swamp bay trees, *Persea palustris* (N = 280), and (B) mean diameter of swamp bay trees and corresponding stage of laurel wilt development, where 0 = asymptomatic, 1 = wilted green to copper leaves (< 1 yr after symptom initiation), 2 = gray leaves (~1 yr), 3 = no leaves (~2 yr), and 4 = fallen tree (~3 yr) (N = 830).

Biological invasion models predict that both host density and host clustering influence disease dynamics. These predictions were tested with data for laurel wilt outbreaks in natural areas in the southeastern US (Er et al., 2013). Considering all hosts, the association of host density with disease spread was non-monotonic, with greater spread at intermediate host density. However, when only hosts with large stem diameters were considered, the correlation was positive and there was a density threshold for invasion. Environmental factors associated with laurel wilt dynamics included daily minimum temperature, daily precipitation and soil organic matter content. Similar analyses have not been conducted for avocado.

Artificial inoculations of different host tree species with *R. lauricola* demonstrate that even small trees (i.e. those that might escape vector-mediated infection in nature) succumb to the disease (Fraedrich, 2008; Fraedrich et al., 2009; 2011; Hughes et al., 2011; 2012; 2013; 2014; Ploetz and Konkol, 2013; Riggins et al., 2011; Smith et al., 2009a; b). However, in avocado studies in the absence of a vector, larger trees were also more susceptible (Fig. 13) (Ploetz et al., 2012b).

Latent infection of artificially inoculated avocado is uncommon, as *R. lauricola* is almost always isolated on microbiological media or detected via qPCR only from symptomatic sapwood.

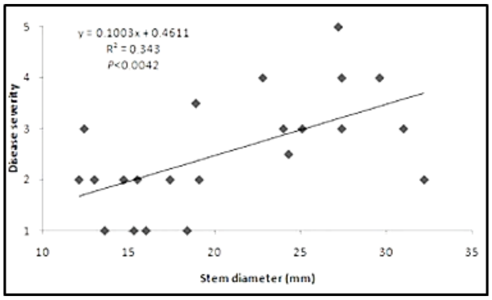


Figure 13. Relationship between stem diameter of 'Simmonds' avocado and the severity of laurel wilt that developed on trees inoculated with *Raffealea lauricola*.

Other possible avenues of infection have been studied experimentally. Mechanical transmission of the pathogen was only possible with artificially infested handsaws on potted plants; the pathogen did not survive on circular saws that are used to prune avocado trees in commercial groves due, perhaps, to the high heat that was generated on these blades during use (Beckman, 2012). Seed and scion transmission of *R. lauricola* have also been discounted. Potted, fruit-bearing trees were artificially inoculated with *R. lauricola* and plants were systemically colonized by the fungus, but in no instance did infection progress further than the hilum of fruit (87 fruit and their associated pedicles) (Ploetz et al., 2012a). And when scions from artificially infected trees were used as grafting material, they did not establish on recipient rootstocks (Ploetz et al., unpublished). Thus, it appears that *R. lauricola* typically infects avocado only via ambrosia beetle vectors and interconnecting root grafts.

IV. Monitoring and Detection

Early detection of laurel wilt, or *R. lauricola*-infected trees before they develop symptoms, is critical to the containment of laurel wilt and the success of disease management efforts in avocado orchards. Monitoring potential vectors in the avocado system is currently inefficient and, thus, not useful for predicting the threat of this disease in a given orchard.

Extensive trapping efforts that target *X. glabratus* are in place in commercial avocado orchards (Carrillo, unpublished). Initial studies that used manuka, phoebe and cubeb oil lures (Hanula et al. 2014; Kendra el al. 2012, 2014b) detected only four individuals of *X. glabratus* from 2012 to 2014 (Carrillo and Kendra, unpublished). Recently, with α-copaene lures that detect low numbers of *X. glabratus* more efficiently (Kendra et al., 2016), the species was detected four times in avocado orchards in 2015 and twice in 2016 (Carrillo and Kendra, unpublished). In the future, α -copaene lures could be important tools for examining the role of *X. glabratus* in the laurel wilt epidemic. Although specific attractants for other potential vectors are not available, ca. 300,000 ambrosia beetles have been trapped using ethanol lures (Miller and Rabaglia 2009) and various essential oil lures in avocado orchards since the beginning of the epidemic (Carrillo, unpublished; Kendra unpublished). Thus, even though trapping these insects is inefficient, the available traps enable research on their relative abundance in avocado orchards and whether a given species carries *R. lauricola*. Current research focuses on detecting *X. glabratus* and other species that carry *R. lauricola* in commercial avocado settings.

Given the potentially confusing symptoms of laurel wilt compared to those associated with other biotic and abiotic factors (see II. Symptoms), the presence of *R. lauricola* should be confirmed when the disease is diagnosed for the first time in a given area or orchard. PCR amplification of ribosomal small-subunit DNA enabled detection of 0.0001 ng of DNA of *R. lauricola* (Dreaden et al., 2008). This approach was suitable for some quantitative PCR applications, but was not taxon specific, as an unidentified *Raffaelea* species was also identified as *R. lauricola* with this method (Dreaden, 2014). The unidentified *Raffaelea* species, recently described as *R. aguacate* sp. nov. (Simmons et al., 2016), is not pathogenic to avocado, redbay or swamp bay (Dreaden et al., 2016). Notably, another method that utilizes ribosomal large-subunit DNA (Jeyaprakash et al., 2014) may also not distinguish *R. aguacate* from *R. lauricola* (Dreaden et al., 2014). Recently, genomic sequencing of *R. lauricola* identified two simple-sequence repeat (SSR) loci that were used to develop a taxon-specific assay (Dreaden et al., 2014). PCR primers that were designed to amplify these loci did not amplify host DNA or DNA from related fungal taxa, including *R. aguacate*. The new SSR-based assay has a detection limit of 0.1 ng of *R. lauricola* DNA, and is compatible with traditional and real-time PCR. Its reliability was confirmed in four different labs, but it could not be used to directly detect the pathogen in wood.

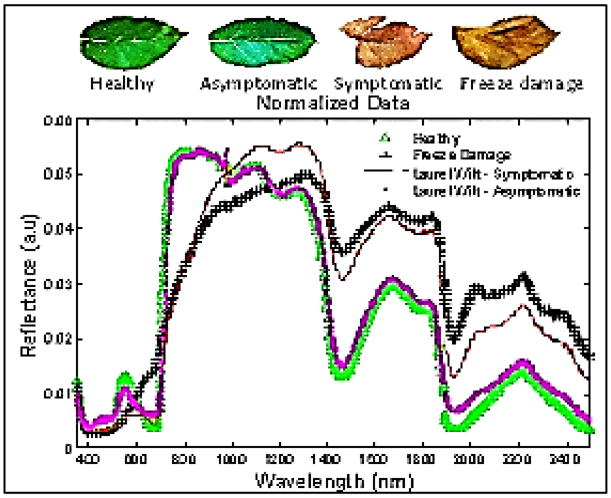


Figure 14. Representative leaves from healthy, *Raffaelea lauricola*-infected (asymptomatic and symptomatic), and freeze-damaged avocado plants. Visible near infrared reflectance spectra representing each category of leaves are shown.

Visible-near infrared spectroscopy was tested for the nondestructive detection of laurel wilt on avocado (Sankaran et al., 2012). Classification studies were conducted with visible-near infrared spectra of asymptomatic and symptomatic leaves from plants artificially infected with *R. lauricola*, as well as leaves from noninfected freeze-damaged and healthy plants (Fig. 14). Scores from principal component analyses were used as input features in four classifiers: linear discriminant analysis, quadratic discriminant analysis (QDA), Naïve-Bayes classifier, and bagged decision trees (BDT). All of the classifiers were able to discriminate leaves from plants with laurel wilt from freeze-damaged leaves. False negatives were primarily for asymptomatic leaves from infected plants that were classified as healthy.

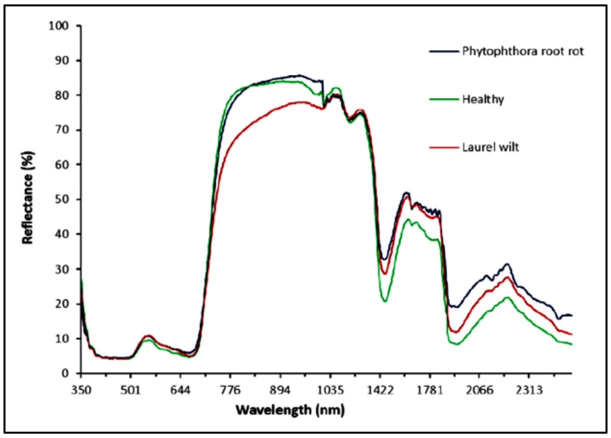


Figure 15. Visible–near infrared reflectance spectra for leaves from healthy (H), laurel wilt-affected (LW) and Phytophthora root rot-affected (PRR) avocado trees.

In subsequent work, spectral data were used to distinguish healthy, laurel wilt-affected, and Phytophthora root rot-affected avocado trees (Castro et al., 2015a) (Fig. 15). With a modified camera, spectral images were taken during helicopter surveys of commercial avocado orchards (Castro et al., 2015b). RmodGB digital data were used to calculate vegetation indices (VIs), band ratios, and VI combinations for healthy and laurel wilt-affected trees. Significant differences were observed in all vegetation indices calculated among laurel wilt affected and healthy trees, although the best results were achieved with Excess Red (ExR), (Red–Green) and Combination 1 (COMB1). These results were used to modify a MCA-6 Tetracam camera with different spectral filters (580–10 nm, 650–10 nm, 740–10 nm, 750–10 nm, 760–10 nm and 850–40 nm), which was then used to take multispectral images of avocado trees at early, intermediate and late stages of laurel wilt development at altitudes of 180, 250 and 300 m (Castro et al., 2015a). At an ideal altitude of 250 m, optimum VIs were calculated using any of the bands related to Redge (740 and 750 nm) or NIR regions (760 and 850 nm). An algorithm that is based on a 4-class system (healthy trees, and those at early, intermediate and late stages of laurel wilt development) is needed to develop an affordable spectral-based detection system for this disease (Castro et al., 2015a).

V. Response

To date, there are no governmental regulations in place that indicate how affected avocado trees should be handled and where infested materials from such trees should be disposed. In the absence of such regulations, it is recommended that affected avocado trees be uprooted and the wood from affected trees be burned or chipped immediately. Wood chips should be spread out and treated with an approved insecticide. The latter activities would depend on local burning regulations and what is labeled for avocado. Recently, an ordinance was proposed in Miami-Dade County that required owners of laurel–wilt affected orchards to dispose of affected trees in a timely manner or risk assessment of a tax lien.

VI. Permit and Regulatory Issues

As of 21 May 2010, firewood could not be moved more than 50 miles from its source in the state of Florida and no firewood or unprocessed wood products could be moved into Miami-Dade County from other areas (see revised rule on Movement of Regulated Articles, 5B-65.005. https://www.flrules.org/gateway/readFile.asp?sid=3&tid=8683166&type=2&file=5B-65.005.htm). The new restrictions should help mitigate the unnatural spread of laurel wilt in Florida.

The Florida Division of Forestry, in cooperation with the University of Florida IFAS, developed a certification program for Florida Pile Burners in 2005 and 2006. In 2009, the Florida Department of Agriculture amended the open burning rules and regulations to include the pile burner certification program. Florida Administrative Code (FAC) 5I-2 outlines the steps necessary to become certified and what is necessary to keep that certification. The rule states that a pile burner maintains their certification if they can show that they have used their certified burn number at least five times in the previous 5 years. In Miami-Dade County, FL, burn permits can be expedited for destroying laurel-wilt affected trees in commercial production areas. The ability to burn affected trees in residential areas in Florida and elsewhere will depend on ordinances in the specific municipality.

VII. Economic Impact

Avocado is a subtropical/tropical tree. Depending on the cultivars that are grown, which vary considerably in their cold tolerance, the crop is grown commercially from USDA Hardiness Zone 10 to 11, with moderate urban production of some cultivars occurring into Zone 9. Commercial avocado production is valued at \$35million year^{-1} (wholesale crop value) in Florida (Evans et al., 2010; Evans and Ballen, 2015) and \$375 million year^{-1} in California (online figures from CA Avocado Commission). In both states, as well as Hawaii and Texas, additional urban production occurs of an unspecified value.

Before laurel wilt impacted commercial avocado plantings in Florida, Evans et al. (2010) estimated that losses could range from \$27-54 million in the absence of effective control measures (50-100% loss). Although effective fungicidal measures are now available, they are expensive. In addition to economic considerations (cost of implementation and the expected benefits, which include treatment efficacy), a practice's adoptability (i.e. the ease with which it can be incorporated into the grower's routine and the time and energy that are expended) should be considered.

Given the cultivars that are currently grown, and yields and prices that are obtained for fruit, some growers have determined that laurel wilt management is uneconomical (costs exceed expected benefits). When these growers are affected by the disease, it becomes increasingly difficult to maintain fruit production. Where little or no management is practiced (i.e. no scouting for laurel wilt development is conducted, laurel wilt-affected trees are not removed quickly and adequately, and fungicides are not applied to impede hotspots development) excessive and rapid loss of trees occurs (see Figs.10 and 11).

VIII. Mitigation and Disease Management

The current strategy for managing laurel wilt in commercial avocado orchards entails prompt detection and destruction of newly affected trees by chipping, treatment of chipped debris with contact insecticides, and protecting surrounding, adjacent trees with macroinfused Tilt fungicide (which has emergency 18C registration) (= "hotspot" treatment) (Ploetz et al., 2016b).

Twenty-eight fungicides in 15 chemical groups and 10 fungicide groups, as defined by FRAC (2016), have been tested for *in vitro* inhibition of *R. lauricola* and reduced development of laurel wilt on avocado (Ploetz et al., 2011b; 2016b; unpublished). Demethylation inhibitors (DMIs; fenarimol, myclobutanil, propiconazole, prothioconazole, triadimenol, triadimefon, and triticonazole), quinone outside inhibitors (azoxystrobin, pyraclostrobin, and fluoxastrobin), and a quinone inside inhibitor (fluazinam) had the greatest impact on radial growth of *R. lauricola* (the concentration at which growth was reduced by 50% was ≥0.1 µg ml–1).

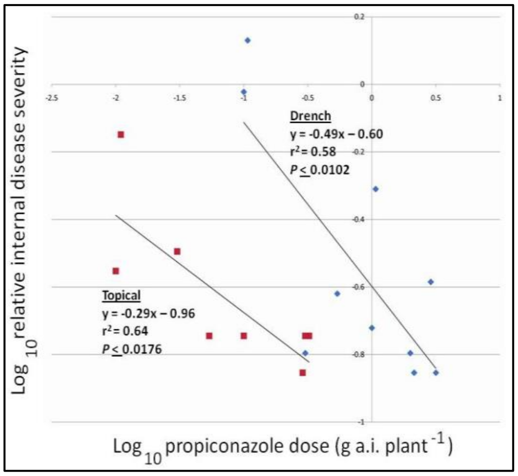


Figure 16. Relative impacts of soil drench (blue diamonds) and topical bark applications (red squares) of propiconazole on laurel wilt development on 'Simmonds' avocado plants artificially inoculated with *Raffaelea lauricola*. Drench applications were in water, and bark applications in 2% Pentrabark. Internal disease severities are relative to (divided by) those that developed on nontreated, inoculated plants in a given experiment. Results from three efficacy experiments were pooled, and each datum represents the mean disease response for five plants at a given propiconazole concentration in one of the experiments.

In greenhouse studies, the most inhibitory products *in vitro*, plus thiabendazole and two products that were not tested *in vitro*, flutriafol and a potassium salts mixture of phosphorus acid, were tested for disease suppression on artificially inoculated, potted avocado trees (Ploetz et al., 2011c). In general, soil drench applications of the above DMIs and thiabendazole, but not azoxystrobin, pyraclostrobin, fluazinam, or the phosphorus acid salt provided significant control of disease ($P < 0.05$). Topical branch or trunk applications of propiconazole, and triadimenol in 2% Pentrabark, a bark-penetrating surfactant, were also effective at lower rates than were used in drench applications. Comparable levels of disease suppression were achieved when propiconazole was applied at 11% of the rates that were used in soil drenches (Fig. 16). Unfortunately, minor concentrations of propiconazole were detected in the xylem of branches of field-grown trees that were larger than ca 2-3 cm in dia, probably due to thick(er) bark impeding penetration and uptake (Ploetz et al., 2016b).

In a recent series of studies in Florida, fungicidal management of laurel wilt was tested in field-grown avocado trees. Trees were treated 2-4 weeks prior to being artificially inoculated with *R. lauricola* (Crane et al., 2015b; Ploetz et al., 2016b). In general, the efficacy of propiconazole was confirmed, although trees were not protected for more than a year in any of the experiments. Although a need for annual retreatment was indicated by the results, it should be noted that minute amounts of the fungicide were detected in fruit (far less than the maximum 2 ppm set by the EPA for food residues), regardless of the application method that was used. Thus, fungicides could be used safely on fruit-bearing trees.

In general, macroinfusion of Propiconazole Pro, an injectable formulation of propiconazole, provided the best protection, although Tilt was also effective if it was applied via macroinfusion (Crane et al., 2015b; Ploetz et al., 2016b). Macroinfusion applies large volumes of dilute fungicide to the flare roots of trees (Blaedow et al., 2010; Mayfield et al., 2008; Stipes, 2000; Tattar, 2007), and in avocado appears to be the most effective way to move fungistatic levels of fungicide to the infection court for *R. lauricola*, the xylem. Unfortunately, macroinfusion is labor-intensive and cannot be used to treat entire orchards due to its expense (Ploetz et al., 2011c). A critical need exists for enhanced application measures, improved formulations of fungicides that would have better mobility in trees, and longer retention times for injected products (Ploetz et al., 2016b). Protecting trees in the field with fungicides is an ongoing challenge.

Due to the importance of *X. glabratus* in natural environments, it was expected that large numbers would attack avocado trees when commercial production areas were affected by laurel wilt. To prepare for this anticipated invasion, extensive efforts were made to develop integrated management tactics for the insect. Peña et al. (2011) and Carrillo et al. (2013) studied the efficacy of more than 20 systemic (Imidacloprid, Emamectin Benzoate, Cyazypyr, Flupyradifurone + Propylen Carbonate, Spirotetramat, Chlorantraniliprole, Sulfoxaflor) and contact (Bifenthrin, Fenpropathrin, Z-Cypermethrin + Bifenthrin, Permethrin, Lambda-Cyhalothrin + Thiamethoxam, Malathion, Chlorpyrifos, Carbaryl, Endosulfan, Novaluron and Diflubenzuron) insecticides. Systemic insecticides applied as a drench did not control *X. glabratus* and when products were injected only Emamectin Benzoate provided partial control of this beetle. Among contact insecticides, Z-Cypermethrin + Bifenthrin, Bifenthin, Fenpropathrin and Malathion significantly reduced the numbers of beetles that bored into avocado logs, but no treatment completely stopped boring by *X. glabratus*. Fenpropathrin, Malathion and Bifenthrin are registered for use in avocado and an emergency exemption (section 18) is in place to use Z-Cypermethrin + Bifenthrin on non-bearing trees. In the field, the above insecticides are also active against other ambrosia beetles that are known to transport *R. lauricola* (Carrillo, unpublished). Once key vectors in the avocado system are identified, chemical control tactics would need to be redirected to target these species.

Three commercial strains of entomopathogenic fungi [two strains of *Isaria fumosorosea* (Ifr 3581 and PFR), and strain GHA of *Beauveria bassiana*] were evaluated recently as biological control agents of *X. glabratus* (Carrillo et al. 2015). Although the strain of *B. bassiana* killed *X. glabratus* females faster than either strain of *I. fumosorosea*, infection by none of the entomopathogens prevented *X. glabratus* from boring into avocado logs; presumably, infected individuals would still infest intact trees with *R. lauricola* if they bored into intact trees.

As noted above for the fungicide studies, insecticide and biopesticide efficacy are constrained by the inherent difficulty of moving products to where they are needed, the host's xylem (ambrosia beetle breeding sites). An additional consideration for vector management is that it would need to be highly effective in order to impact laurel wilt development, as a single interaction of a *R. lauricola*-infested ambrosia beetle with a healthy avocado tree may be all that is needed to initiate a lethal infection. Whether treatments that would be less than totally effective against vectors would still have an impact on laurel wilt development needs to be determined.

Persea americana is divided into Mexican (M), Guatemalan (G) and West Indian (WI) (aka Lowland or Antillean) botanical races (Lahav and Lavi, 2013). From 2010 to 2012, 24 commercial avocado cultivars of various racial backgrounds were screened for response to laurel wilt in field studies (Ploetz et al., 2012b). Although resistant cultivars were not identified, G and MxG hybrid cultivars were more tolerant than WI cultivars, such as 'Simmonds,' which comprises 35% of the commercial production in Florida and has been used as a standard susceptible genotype in different studies. Mechanisms that were associated with the range of responses that were evident in these trials were not examined. For example, the extent to which xylem function and other factors may have influenced disease development in the different cultivars was not assessed. To date, mature trees of 28 avocado cultivars (nine WI, 14 WI x G, two of unknown parentage and one G, GxMxWI, and GxM cultivar) have been killed by laurel wilt in the CAPA (Crane et al., 2015a).

Developing new scion genotypes that resist laurel wilt and also possess desirable organoleptic and horticultural traits would be a long-term process. Current research examines the impact of different rootstock and scion combinations on xylem attributes and laurel wilt susceptibility. In other crops, rootstocks can significantly influence the grafted scion. For example, different avocado rootstocks affected post-harvest disease development on fruit of 'Hass' (Willingham et al., 2006), and xylem sap flow rates in 'Hass' scions (Fassio et al. 2009).

Commercial avocado scions are traditionally grafted on seedling WI rootstocks in Florida, which are quite variable due to their hybrid nature (avocado preferentially outcrosses). Given the positive relationship between xylem sap flow rates and laurel wilt susceptibility (Fig. 17), the influences that rootstocks have on scions, and the variable laurel wilt responses that have been observed when clonal avocado scions are grafted on seedling rootstocks (note variation in Fig. 13), Ploetz et al. (2015) proposed that clonal avocado rootstocks could influence the susceptibility of a given scion to laurel wilt; they suggested that less susceptible G and M genotypes would reduce the susceptibility of WI scions when used as rootstocks, and that clonal, rather than seedling, rootstocks should enable a more consistent scion response.

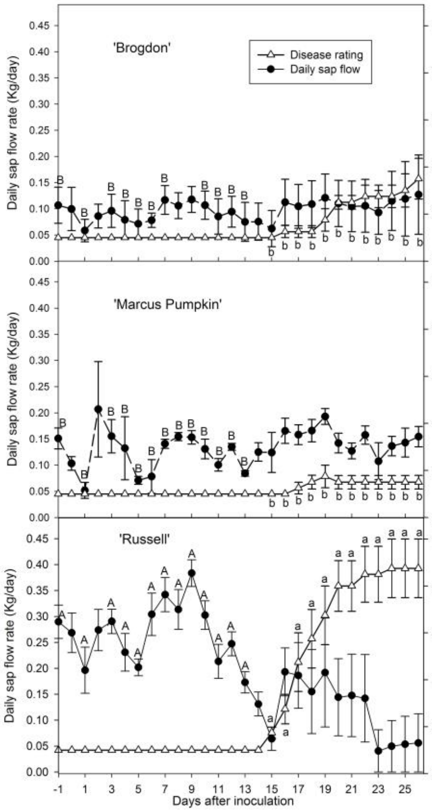


Figure 17. Mean daily xylem sap flow and visual disease rating for 'Brogdon' (MxGxWI hybrid) 'Marcus Pumpkin' (G) and 'Russell'(WI) avocado trees inoculated with *Raffaela lauricola*. Different upper case letters indicate significant difference in xylem sap flow among cultivars and different lower case letters indicate significant differences in disease rating among cultivars according to a repeated measures ANOVA ($P < 0.05$). Absence of letters indicate no significant differences between cultivars ($P > 0.05$). Note significantly higher sap flow rates in laurel wilt susceptible 'Russell', compared to 'Brogdon' and 'Marcus Pumpkin'.

IX. Infrastructure and Experts

Pathology, Mycology

Tyler Dreaden, Research Pathologist, USDA-Forest Service, Lexington, KY, conducted research on the molecular characterization of ambrosia beetle symbionts and the detection of *R. lauricola* as a graduate student at UF. He continues work on laurel wilt in his current position.

Stephen Fraedrich, Research Pathologist, Forest Service, Athens, GA, was the first to identify laurel wilt. He has documented its impact on native lauraceous hosts, examined its host range, and investigated vector relationships with *X. glabratus* and other ambrosia beetles.

Marc Hughes, post-doctoral in the School of Forest Resources and Conservation, UF, Gainesville, has considerable experience with laurel wilt on native trees, especially redbay. He led the revision of the recovery plan for laurel wilt on native Lauraceae that was published in 2015.

Randy Ploetz, Professor of Plant Pathology, University of Florida (UF) in Homestead, is an authority on the diagnosis and management of tropical fruit diseases, including those that impact avocado. He currently researches host responses and resistance to laurel wilt, laurel wilt management, and the host range and alternative vectors of *R. lauricola*.

Entomology, Vector Relations

Daniel Carrillo, Assistant Professor of Entomology, Entomology and Nematology Department, UF, Tropical Research and Education Center, Homestead, is an expert in Tropical Fruit Entomology and studies the ambrosia beetles associated with the laurel wilt epidemic in South Florida.

Jiri Hulcr, Assistant Professor of Forest Entomology, School of Forest Resources and Conservation, UF, Gainesville, is an expert on ambrosia beetles, their symbionts, and their host interactions.

Paul Kendra, Research Entomologist, USDA-ARS Subtropical Horticulture Research Station, Miami, conducts research on host-based attractants for *Xyleborus glabratus* and lure development.

Bud Mayfield, Research Entomologist, USDA Forest Service, Southern Research Station, Asheville, NC, has considerable experience monitoring the spread of *X. glabratus* and laurel wilt, and assessing the attractiveness of avocado and other hosts to *X. glabratus*.

Lukasz Stelinski, Associate Professor of Entomology, Entomology and Nematology Department, UF, Citrus Research and Education Center, Lake Alfred, is a chemical ecologist and an expert in insect-vectored plant diseases, in particular huanglongbing of citrus.

Extension

Jonathan Crane, Professor of Horticultural Science, UF, Tropical Research and Education Center, Homestead, is an expert on the production systems of tropical fruit crops including avocado. His extension program addresses the management of laurel wilt.

E.A. Evans, Professor of Food and Resource Economics, UF, Tropical Research and Education Center, Homestead, investigates the economic impact of laurel wilt and cost considerations for its management.

Administration

Julius Fajardo, Plant Pathologist, Office of Pest Management Policy, USDA, Washington, D.C.

X. Research, Extension and Education Priorities

Cost-effective management of laurel wilt on avocado will require new information in several key areas. The ambrosia beetle species that are vectors of this pathogen (i.e. initiate infection in healthy trees as opposed to colonize diseased and dying trees) should be determined. The available evidence suggests that resident ambrosia beetles are vectors in the avocado pathosystem, but that they are inefficient. Little is known about the fundamental biology of these previously inoffensive species. Information on the reproductive potential, seasonality, and flight behavior of these species in avocado groves is needed, as it could provide essential insight into their roles in the development of this disease on this important host tree. To understand the role that these species play in the spread of laurel wilt, it may also be necessary to understand their responses to symbionts and endophytes that they would encounter in avocado trees.

The radical response of susceptible hosts to inoculation with *R. lauricola* is poorly understood. More information is needed for how the pathogen interacts with different host species and different avocado cultivars, as well as the fundamental mechanisms of transmission. Defining acquisition and inoculation periods, as well as latency and vector capacity, among the various species that transmit *R. lauricola* to avocado should improve insecticidal and cultural management practices for the vectors. Nothing is known about pathogen products that are produced *in planta* that impact disease development. Likewise, it is not clear whether there are differences in the extent and distribution of colonization by *R. lauricola* in tolerant and susceptible hosts, as was suggested by preliminary work (Fig. 5). For example, whether systemic colonization is associated with susceptibility and restricted development of the pathogen is associated with tolerance should be determined. Indirect effects of the pathogen on the vector via plant responses, as well as plant defense mechanisms against the vector, are also still poorly understood.

In response to stakeholder surveys, extension methods to inform and demonstrate management strategies for laurel wilt have shifted to provide more on-line content in the form of videos, PowerPoints, email and literature. Workshops, seminars and field demonstrations of limited subject matter and of shorter duration are desired by stakeholders.

XI. Timeline for Recovery

Approximately 2% of the commercial avocado trees in the CAPA have been lost to laurel wilt. Some producers of limited economic means have dropped out of the industry, and more may follow as the disease spreads. Nonetheless, producers that manage about 60% of the industry have replanted avocado in areas where mature trees have been killed by the disease.

Resiliency factors that impact recovery:

- Multiple vectors may be involved. At best, our ability to manage vectors is incomplete, even in a local sense. Since the pathogen is thought to be successfully transmitted to avocado after a single interaction with a vector, management of laurel wilt via vector control will be difficult.

- The pathogen can colonize and quickly kill avocados, and focal spread of the disease by root graft transmission is rapid in avocado orchards. Reducing or eliminating focal spread is a great challenge.

- Substantial reservoirs of the pathogen and vector(s) exist in natural areas in affected and threatened avocado-production areas. Based on the available evidence, it appears that the reservoirs play an important role in laurel wilt establishment in a given area. Thus, development of effective management strategies for laurel wilt in natural ecosystems and residential landscapes adjacent to the CAPAs would complement management efforts within orchards. Local populations of native hosts (especially redbay and swamp bay) have been devastated by laurel wilt. The impact that decreased reservoir numbers will have on corresponding disease pressure in neighboring avocado productions areas is unclear.

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Web Resources

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- http://www.ambrosiasymbiosis.org/researchers/jiri-hulcr/
- https://www.invasivespeciesinfo.gov/microbes/laurelwilt.shtml UF/IFAS TREC laurel wilt of avocado: http://trec.ifas.ufl.edu/RAB-LW-2/index.shtml (a new site is under construction)
- FDACS Save the Guac: http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Save-the-Guac
- FDACS laurel wilt disease: http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Pests-Diseases/Laurel-Wilt-Disease