Recovery Plan
for
Laurel wilt of Avocado

(caused by *Raffaelea lauricola*)

22 March 2011

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 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.

Executive Summary

Laurel wilt kills American members of the Lauraceae plant family, including avocado (*Persea americana*). The disease threatens commercial avocado production in Florida, as well as the National Germplasm Repository for avocado in Miami (USDA-ARS). Elsewhere in the US, major (California) and minor commerce in the fruit (Texas, Hawaii and Puerto Rico) could be impacted if the disease continues to spread.

Laurel wilt is caused by a recently described fungus, *Raffaelea lauricola*, which has an Asian ambrosia beetle, *Xyleborus glabratus*, as a vector both in Asia and the USA. *X. glabratus* originated in Asia and was reported for the first time in the Western Hemisphere in May 2002 in Port Wentworth, GA, a maritime port near Savannah. Shortly afterwards, laurel wilt was observed in the vicinity on redbay, *Persea borbonia*, a dominant component of Coastal Plain forest communities in the southeastern USA. Redbay has been devastated in the ensuing epidemic, and a separate recovery plan for it and other native forest suscepts has been written.

The first avocado trees were killed by laurel wilt in 2006 in Duval County, FL, and the disease has been documented on avocado as far south as Brevard County, FL. Virtually all commercial avocado production in Florida is centered in Miami-Dade County, ca 200 km south of Brevard County. In February 2011, laurel wilt was confirmed on swampbay, *P. palustris*, in Miami-Dade County, about 3 km north of the nearest commercial avocado production area.

In general, American members of the Lauraceae are more susceptible to the disease than are those from the beetle's Asian home range. Host and *X. glabratus* interactions are less clear. Scant information is available on the extent to which lauraceous and non-lauraceous taxa serve as hosts and reservoirs for the vector and pathogen.

Laurel wilt has spread due to its mobile insect vector, the movement of infested wood, and the presence of native and non-native plants throughout the southeastern USA that are susceptible to the disease and on which the vector reproduces. Rapid spread has occurred where there are high population densities of redbay and swampbay (some taxonomists do not distinguish these species). In avocado, movement of the pathogen by root grafts is probable and by pruning equipment is possible; the possibilities of movement via fruit, seed or scion material are under investigation.

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, chemical mitigation and cultural measures will be needed. In the latter situation, the prompt identification and removal of infected trees (sanitation) before emergence of brood will probably play a significant role; sanitation will rely on rapid and specific means by which laurel wilt could be diagnosed.

The Southern Region of the USDA Forest Service maintains a frequently updated website on diverse topics related to laurel wilt (http://www.fs.fed.us/r8/foresthealth/laurelwilt/index.shtml). UF/IFAS Extension and FDACS-DPI have informed commercial, governmental and urban clientele in Florida through in-person and electronic workshops, seminars, the development and dissemination of extension publications, and presentations and publications posted on websites (e.g., http://www.freshfromflorida.com/pi/enpp/pathology/laurel_wilt_disease.html and http://edis.ifas.ufl.edu, http://trec.ifas.ufl.edu).

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 needed, used, and effective in commercial avocado-production areas.

1. Research on the efficacy and cost-effectiveness of chemical controls measures (fungicides, and insecticides, attractants and repellents of *X. glabratus*) must continue as they may ultimately provide important components of a multifaceted management scheme for laurel wilt. Likewise, 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/genotypes will probably be needed if resistance is to play a significant role in addressing this disease.

2. A reliable and rapid diagnostic test for *R. lauricola* is an urgent need. Its development and implementation in newly affected and threatened areas should continue, as it will be an important tool for identifying trees in sanitation efforts.

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 whether they might also spread the disease. Likewise, nothing is known about the spread of *X. glabratus*, *R. lauricola* and laurel wilt in avocado monocultures (i.e. commercial production groves); understanding these events will be especially important once native hosts (e.g. redbay and swampbay) are eliminated and avocado is the sole, remaining susceptible taxon in a given area.

4. State and federal 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 SE USA and will likely result in the continued spread of this disease. Regulations recently enacted in Florida (see VI. Permit and Regulatory Issues) provide useful models for what could/should be considered in other states.

5. State and federal efforts to educate stakeholders on the disease and its mitigation should continue.

Laurel Wilt of Avocado

(caused by *Raffaelea lauricola*)
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I. Introduction

Laurel wilt is caused by a fungal symbiont, *Raffaelea lauricola* (Figs. 1A and 1B), of the redbay ambrosia beetle, *Xyleborus glabratus* (Figs. 2A, 2B and 2C), which is the only known vector of the pathogen (Fraedrich et al. 2008). Laurel wilt kills American members of the Lauraceae plant family, including avocado, *Persea americana*. The disease threatens commercial avocado production in Florida, centered in Miami-Dade County (\$54 million yr^{-1}), as well as the National Germplasm Repository for avocado in Miami (USDA-ARS) (Evans et al., 2010). Elsewhere in the US, major (California, \$342 million in 2006) and minor commerce in the fruit (Texas, Hawaii and Puerto Rico) could be impacted if the disease continues to spread.

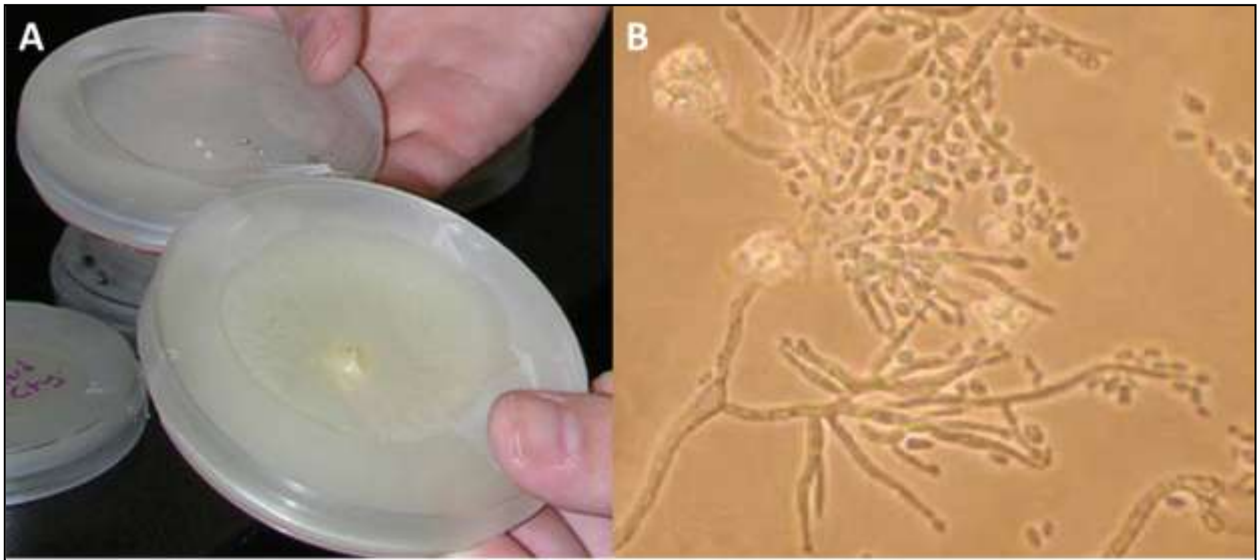


Figure 1. A) Colonies of *Raffaelea lauricola* on CSMA (Ploetz), and B) conidia and cpnidiophores of the fungus produced in culture (courtesy of S. Fraedrich).

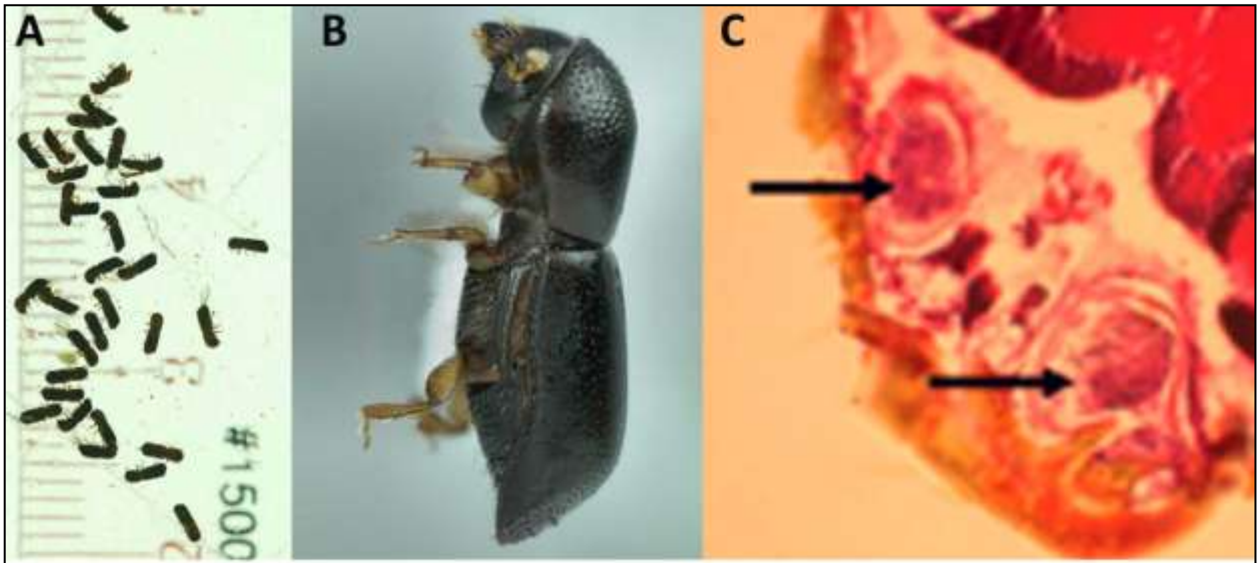
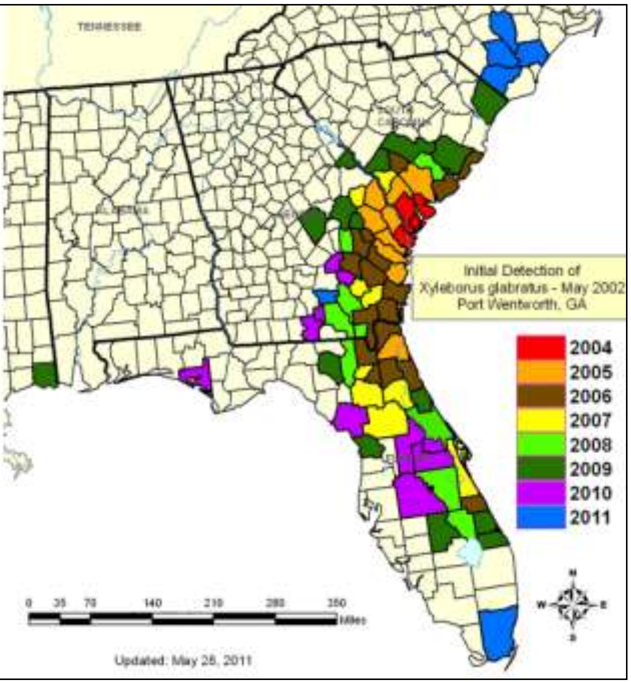


Figure 2. A) Adults of *Xyleborus glabratus* on a metric ruler (courtesy of Robert Rabaglia), B) lateral view of an adult female of *X. glabratus* (courtesy of Mike Thomas), and C) cross-section of mycangia of an adult female of *X. glabratus* (courtesy of Mike Ulyshen); note that the mycangia are filled with fungal spores.

Due to the natural dispersal of *X. glabratus* and movement of infested wood, laurel wilt has spread rapidly along the southeastern seaboard of the USA (USDA Forest Service 2011). Redbay and other native species in the Lauraceae have been affected, and several non-native members of the family have also been impacted or shown to be susceptible after artificial inoculation (Fraederich 2008, Mayfield et al. 2008c, 2009, Smith et al. 2009a, 2009b).

Figure 3. Spread and distribution of laurel wilt in the southeastern USA, as of May 2011. The initial outbreak of laurel wilt and much of its spread from the Savannah area has been on the native tree, redbay (*Persea borbonia*). The 2011 outbreak in southeastern Florida (Miami-Dade County) was on swampbay (*P. palustris*) but, as of July 2011, had not impacted commercial avocado production that was located in the area. This map is located at: http://www.fs.fed.us/r8/foresthealth/laurelwilt/index.shtml; was complied with information from Laurie Reid, Jeff Eickwort, Chip Bates, John J. Riggins, and Rob Trickel; and is updated periodically.



As of April 2011, laurel wilt had been recognized as far north in the USA as Sampson County, North Carolina (35° N), as far south as Miami-Dade County, Florida (25.7° N), and as far west as

Jackson County, MS (ca. 88.7^{o} W) (Ploetz et al. 2011a, Riggins et al. 2010, USDA Forest Service 2011) (Fig. 3). The Miami-Dade outbreak is 3 km to the north of Florida's commercial production areas for avocado.

In 2006, avocado seedlings (unspecified cultivar) succumbed to artificial inoculation with *R. lauricola* in a growth chamber trial (Fraedrich et al. 2008), and in 2007 the first naturally affected avocado tree (unknown cultivar) was reported in the Jacksonville area (Mayfield et al. 2008c). Residential avocado trees have continued to die as the pathogen moved south in the state, but as of April 2011 Florida's commercial avocado production area had not been impacted.

About 3.5 million metric tons (MMT) of avocado were harvested worldwide in 2008 (FAOSTAT). Mexico was the most important producing country (1.1 MMT) and the USA ranked ninth. California and Florida are the primary producing states in the USA (Evans 2008).

Three botanical races of avocado are recognized (Knight 2002). The Mexican (M) (*P. americana* var. *drymifolia*) and Guatemalan (G) (*P. americana* var. *guatemalensis*) races originated in the respective highlands of those countries, whereas the West Indian (WI) or Antillean race (*P. americana* var. *americana*) arose on the Pacific coast of Central America. Due to environmental adaptations and marketing histories, different cultivars are grown in California and Florida: M, G and MxG hybrid cultivars predominate in California, and WI, G and WIxG cultivars are most important in Florida (Knight 2002). One MxG cultivar, 'Hass,' accounts for 95% of all production in California, but 23 major and 20 minor cultivars are produced in Florida. Commercial production in both states relies on clonal scions that are grafted on clonal or seedling (most common) rootstocks. Commercial production in California occurs in four counties (Riverside, San Diego, Santa Barbara and Ventura), whereas that in Florida is highly localized with 99% in southeastern Miami-Dade County. In 2007/08, 165,000 tons of fruit worth $328 million were harvested in California, and 27,500 tons of fruit worth $12 million were harvested in Florida (Evans 2008, USDA).

Raffaelea lauricola is a typical ambrosia symbiotic fungus that is tightly associated with *X. glabratus* in Asia and the USA (Harrington and Fraedrich 2010, Harrington et al. 2011). *Raffaelea lauricola* is closely related to other symbiotic ambrosia fungi in the anamorphic genus *Raffaelea* (Harrington et al. 2008). This genus is phylogenetically placed in the Ophiostomatales (Alamouti et al. 2009, Gebhardt and Oberwinkler 2005, Harrington et al. 2010), The Ophiostomatales includes a few plant pathogens, such as *O. novo-ulmi*, the cause of Dutch elm disease, and *Leptographium wageneri*, the cause of black stain root disease on conifers (Harrington 2005). *R. lauricola* is the only ambrosia beetle symbiont that also is a plant pathogen (Harrington et al. 2010). In a revision of ambrosia beetle symbionts (Harrington et al. 2010), those symbionts with *Ophiostoma* affinities were placed in *Raffaelea*, *Dryadomyces* was synonymized with *Raffaelea*, and *Ambrosiella* species were restricted to ambrosia beetle symbionts with *Ceratocystis* affinities in the Microascales. Phylogenetic analyses by Alamouti et al. (2009) suggested that there were two subgroups within *Raffaelea* that, if separated as two anamorphic genera, would transfer *R. lauricola* to another genus. Harrington et al. (2010), however, retained both subgroups within *Raffaelea* because they do not differ in biology or morphology.

Females of *X. glabratus* are responsible for dispersing the pathogen, since males are flightless. *Raffaelea lauricola* is transported in specialized structures within *X. glabratus*, mycangia, that are located behind the insect's mandibles (Fraedrich et al. 2008). 'Gardens' of *R. lauricola* are produced in galleries of the insect in affected trees, and they are presumed to be a primary food source for the beetle. Harrington et al. (2010) described several other relatives of *R. lauricola* in the mycangia of *X. glabratus* in the USA, and some of these symbionts were also associated with the beetle in Taiwan and Japan (Harrington et al. 2011). However, *R. lauricola* predominates and the significance of the other *Raffaelea* spp. to the biology of the beetle is not known. It is speculated that in both the USA (Fraedrich et al. 2008) and in Asia (Harrington et al. 2011), *R. lauricola* is introduced by *X. glabratus* females as they bore into healthy trees, but such galleries are not used for brood development until other female *X. glabratus* excavate these brood galleries in trees after *R. lauricola* has moved systemically in the plant and caused wilt (Harrington et al. 2011).

How *X. glabratus* locates potential host trees is incompletely understood. Surprisingly, *X. glabratus* does not appear to be significantly attracted to ethanol, which is a general attractant for most ambrosia beetles (Hanula et al. 2008). Host volatile compounds may play an important role in long-range attraction (Hanula and Sullivan 2008, Hanula et al. 2011), whereas other factors could play roles in short-range attraction and interactions. For example, α-copaene appears to be a significant component of the semiochemical signature of redbay (Hanula and Sullivan 2008) and other preferred host and non-host trees (e.g. lychee, *Litchi chinensis*) (Kendra et al. 2011). Other factors, such as the odor of fungal symbionts, may constitute short-range cues with some ambrosia beetles (Hulcr et al. 2010); however, trap catches of the beetle were no higher in redbay logs infested with *X. glabratus* and *R. lauricola* than in *R. lauricola* uninfested logs (Hanula et al. 2008).

Likewise, the host x pathogen interaction is only beginning to be understood (Inch et al. 2011, Inch and Ploetz 2011). As disease severity increases in inoculated avocado plants, water conduction and functional xylem are reduced. After artificial inoculation, the pathogen spreads rapidly throughout the plant and can be reisolated from locations above and below the inoculation point on a semi-selective medium (Harrington, 1981) (CSMA). Low titers of pathogen DNA are detected via qPCR in infected plants and, based on histological examinations, relatively few xylem vessels are colonized. Hydraulic impedance appears to result from the formation of tyloses and gels in xylem vessels rather than plugging by the pathogen (Inch and Ploetz 2011).

II. Symptoms

Many of the symptoms of laurel wilt on avocado resemble those caused by other diseases or abiotic factors. Lightening damage can directly kill canopies of trees, whereas other diseases can do so indirectly. All or portions of trees that are affected by Phytophthora root rot, caused by *Phytophthora cinnamomi*, and Verticillium wilt, caused by *Verticillium dahliae*, wilt and eventually die. Vascular discoloration similar to that caused by laurel wilt also develops with Verticillium wilt.

The first external, foliar symptoms of laurel wilt on avocado are wilting of terminal leaves that change from an oily green color to brown soon after wilting (Fig. 4A). Symptoms typically develop rapidly in affected portions of the tree, but systemic development in which the whole tree dies is inconsistent in avocado (Fig. 4B). The production of healthy branches beneath affected regions in the tree (Fig. 4C) or the unilateral development of symptoms in which only a branch or a portion of a tree are affected also may occur. Unlike laurel wilt-affected redbay, which retains dead leaves for a year or longer, avocado usually defoliates within 2-3 months of

Figure 4. A) Initial, oily green symptoms of laurel wilt on avocado, B) sectoral symptom development in which only a portion of an avocado tree is affected, C) defoliation 2 months after the initiation of symptoms (note production of healthy, epicormic branches beneath the affected area, and D) internal vascular discoloration in avocado caused by the disease. A) to C) are 'Simmonds' trees that were artificially inoculated with *Raffaelea lauricola* in disease screening trials.

symptom initiation (Fig. 4C). Internally, affected avocado sapwood is discolored reddish brown to bluish grey (Fig. 4D).

In artificial inoculation studies, moderate internal symptoms develop on avocado before external symptoms become evident (Ploetz et al. 2011b). On a 1-10 scale, where 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 severities may exceed 5 before external severities are greater than 1. The internal/external symptom threshold has important implications for disease management since it is doubtful that current formulations of systemic fungicides or insecticides would be very mobile or effective if they were applied after external symptoms began to develop. Fungicides are not effective against Dutch elm disease if treatment occurs after more than 20% of the canopy is affected (Stipes 2000).

III. Spread

Much of what is known about the epidemiology of laurel wilt comes from work on North American hosts in natural landscapes. Although other avenues of dissemination may be possible (see below), it appears that *R. lauricola* spreads solely or primarily via its ambrosia beetle vector, *X. glabratus.* Thus, Harrington and Fraedrich (2010) suggested that management of the disease should focus on managing populations of the vector. Advances in the management of this disease should come from improved understandings of the vector's movement, how it identifies host trees, and how and why it ultimately colonizes and establishes broods in avocado and other host trees.

Koch and Smith (2008) used climate matching (ecological niche modeling based on a species' native range) and the geographic distributions and densities of two laurel wilt suscepts, redbay and sassafras (*Sassafras albidum*), to model the spread of *X. glabratus*. They predicted that *X. glabratus* would reach Miami-Dade County by 2015. However, 5 years before this date, a female of *X. glabratus* was trapped in northern Miami-Dade County (2 March 2010; 25.75900N, 80.43455W).

The extent to which factors that were not considered by Koch and Smith (2008) play a role in the movement of *X. glabratus* is not known. For example, other suscepts, such as swampbay and avocado, were not considered in their model, nor was the anthropogenic movement of affected host materials.

Host choice by the beetle vector is still poorly understood, despite being one of the most important factors in the spread of this pathogen. Unfortunately, information on host choice by the beetle in its native habitat (Southeast Asia) is of little help for management purposes since the available hosts are different in the invaded region (North America). Although the beetle has not been associated with tree mortality in its native region (Beaver and Liu 2010), Harrington et al. (2011) speculated that the beetle in Asia uses the pathogen to kill branches or whole trees for brood material, as it does in the USA (Fraedrich et al. 2008). In North America the beetle has only been recovered from freshly killed trees. Host choice in North America will be a critical focus of future research, as this will: 1) determine the rate of disease spread in different ecosystems, 2) influence whether the vector and pathogen establish long-term associations with avocado and alternative hosts, and 3) impact the success of disease mitigation on avocado.

Preliminary results (see below) indicate that high population densities of the beetle are maintained as long as susceptible, preferred hosts are available. Fortunately, avocado appears to be a less preferred host. Thus, avocados may be sinks for the beetles whereas the preferred host species are sources of new generations of the vector.

Silkbay, *P. humilis*, was significantly more attractive to *X. glabratus* than were avocado and three other lauraceous natives, redbay, swampbay, and lancewood (*Ocotea coriacea*) (Kendra et al. unpublished data). Avocado attractiveness was not significantly different from that for the other lauraceous hosts. Hanula et al. (2008) and Hulcr, Stelinski and Mann (unpublished data) also observed that *X. glabratus* was attracted to both avocado and redbay; however, Hulcr, Stelinski and Mann (unpublished data) indicated that redbay was preferred over avocado. Anecdotal data on Merritt Island (Brevard County, FL) corroborate these observations, in that relatively few avocado have been affected by laurel wilt when surrounded by dead and dying redbay infested with *X. glabratus*.

More information is needed on the interaction of *X. glabratus* with these and other host and non-host taxa. Likewise, a better understanding of how the presence and prevalence of various laurel wilt suscepts in a given area impact the development of local epidemics. For example, although *X. glabratus* interacts with avocado, that may occur only after most of the redbays suitable as brood trees have been eliminated in an area (Hulcr, Stelinski and Mann unpublished data).

The lower attraction of the vector to avocado seems to explain why Hanula et al. (2008) detected fewer entrance holes in avocado than in redbay. The ability of *X. glabratus* to propagate in infested trees may also vary among host taxa. In this regard, avocado appears to be a relatively poor host in that only 4 of 1,000 adult scolytids that emerged from laurel wilt-affected avocado bolts were *X. glabratus*, whereas 980 of 1,000 that emerged from redbay were of this species (Peña et al. unpublished). Although reasons for these results are not clear, they could have important implications for whether laurel wilt epidemics could develop in avocado in the absence of redbay or other more preferred hosts.

In general, ambrosia beetles were presumed to have close associations with one or only a few symbiotic fungi (Batra 1967). However, the recovery of six different species of *Raffaelea* from *X. glabratus* by Harrington and Fraedrich (2010) indicates that this presumption may be incorrect, at least for this species. Moreover, symbionts are not necessarily restricted to a single species of ambrosia beetle (Batra 1967) and the lateral transfer of a symbiont species from one beetle species to another has been reported (Gebhardt et al. 2004). Thus, Harrington and Fraedrich (2010) and Kendra et al. (2011b) suggested that *R. lauricola* might have ambrosia beetle vectors other than *X. glabratus*. Ott (2007) determined that *R. lauricola* did not establish in the mycangia of *Xylosandrus crassiusculus*, but it has been recovered from adult *Xyleborinus saxeseni* (Ratzeburg) (Fraedrich et al. 2011), *Xyleborus affinis* and *Xyleborus ferrugineus* (Ploetz et al. unpublished data). To date, the infestation and inoculation of healthy avocado, redbay or other hosts by these ambrosia beetle species has not been demonstrated.

More work is needed to clarify the significance of these findings and whether additional beetle species might also obtain and transmit this pathogen. The unique relationship between *R. lauricola* and *X. glabratus* in Asia may indicate that only *X. glabratus* would be an important vector (Harrington et al. 2011). Since they usually do not infest living trees, ambrosia beetles other than *X. glabratus* that are colonized by *R. lauricola* may not pose a threat to avocado; however, outside the native range of *X. glabratus*, they may become reservoirs for *R. lauricola*. The role of ambrosia beetles other than *X. glabratus* in the persistence of this pathogen in newly infested areas is an important topic for future study.

Other types of pathogen dissemination are possible and need to be considered. The movement of infested material by humans has been one of the main routes for long range dispersal, but it is difficult to quantify, predict and document. Although a hobbyist evidently spread the disease by moving a laurel-wilt-affected redbay log from Jacksonville to Daytona Beach, Florida (ca 120 km) (Chemically Speaking Feb 2009), most examples of anthropogenic spread can only be surmised from dramatic jumps in the disease’s distribution; for example, 550 km to Mississippi (Riggins et al. 2009) and 140 km to Miami-Dade County (Ploetz et al. 2011a). Recent restrictions that were placed on the movement of firewood in Florida may help reduce the occurrence of such events within that state (see VI. Permit and Regulatory Issues).

Ongoing research indicates that chipping infested materials dramatically reduces the survival of both the vector and pathogen (Spence and Smith, unpublished). Thus, chipping of affected materials onsite might be an effective first step in disposing of such materials.

Root-graft transmission occurs for similar vascular wilt diseases, such as Dutch elm disease. For these diseases, functional root grafts between healthy and affected trees are severed by trenching to impede tree-to-tree spread. Root grafting is known to occur between avocado trees and circumstantial evidence indicates that root-graft transmission of *R. lauricola* probably occurs in avocado and redbay (Ploetz and Smith, unpublished observations).

Mechanical transmission of *O. novo-ulmi* to American elm occurs via pruning equipment, and may occur for *R. lauricola* to avocado and redbay (Beckman and Smith, unpublished observations). Work is underway to identify noncorrosive products for disinfesting pruning equipment that is used during topping and hedging operations in laurel wilt-affected avocado orchards.

About 80% of the avocado fruit that are produced in Florida are sold outside the state, and avocado seed are exported from Florida for rootstock production in California, the Dominican Republic and other producing areas. Thus, there is concern that fruit and seed from laurel wilt-affected trees might also disseminate *R. lauricola* to areas that are currently free of laurel wilt. Recent work on artificially inoculated avocado trees suggests that avocado fruit and seed are not infected by *R. lauricola* (Ploetz et al. unpublished). On a semi-selective medium, the pathogen was always recovered from branches that supported fruit and usually from the supporting pedicel. However, it was never recovered from the hilum, fruit flesh, seed, or cotyledons of assayed fruit. Furthermore, the pathogen was not detected in the latter tissues with a sensitive quantitative polymerase chain reaction (qPCR) assay (Dreaden et al. 2008). In addition to repeating these studies, similar research will be conducted to determine the potential for disseminating infected scion material, either in grafted plants or via budwood.

The aerial movement of *R. lauricola* without *X. glabratus* is improbable, as is the establishment of the pathogen in a new area without its vector. The fungus produces wet masses of thin-walled, single-celled spores that are sensitive to desiccation (reduced survival after 8 hours of air drying and none after 48 hours) (Ploetz, unpublished). Even if aerial dissemination of the pathogen were possible, it is unclear how it would infect trees given the xylem infection court and high levels of the pathogen that are introduced by *X. glabratus* in natural infections (Harrington and Fraedrich 2010).

IV. Monitoring and Detection

Surveys for laurel wilt on native host species began shortly after redbay began to die in Georgia and South Carolina (Mayfield et al. 2009). The first avocados were killed by laurel wilt in 2006 and 2007, but specific surveys for the disease on this host only began in Miami-Dade County when the disease was reported there in July 2009. That erroneous report resulted in a considerable effort by the Florida Department of Agriculture and Consumer Services (FDACS) Division of Plant Industry (DPI) and the USDA's Animal and Plant Health Inspection Service (APHIS) Cooperative Agricultural Pest Survey (CAPS) to identify the disease and trap *X. glabratus* in Miami-Dade County. The latter efforts yielded a single female of *X. glabratus* in early 2010, but no reports of the disease.

In February 2011, laurel wilt was confirmed for the first time in Miami-Dade County on swampbay trees (Ploetz et al. 2011a). Subsequent aerial surveys identified an elliptical disease focus extending ca 14 km from north to south. Defoliation of some of the trees in the focus center suggested that laurel wilt may have been in the area for at least a year. As of April 2011, the disease was about 3 km from the nearest commercial avocado orchards. Additional surveys were planned to delineate the extent of the outbreak.

Given the ambiguous symptoms that laurel wilt causes on avocado, *R. lauricola* must be confirmed in suspect samples to accurately diagnose this disease. CSMA is useful for routine isolations and *R. lauricola* generally produces characteristic colonies on it when isolations are made from surface-disinfested, affected host tissue. Since other fungi can grow on CSMA, isolates that resemble *R. lauricola* should be examined further. Relying solely on the appearance of colonies on this medium should not be used as a final step in diagnosis.

Dreaden et al. (2008) developed PCR primers to amplify small subunit (SSU) ribosomal DNA of *R. lauricola* (see SOPs). With the primers, conventional and quantitative PCR identifications were possible for cultures of *R. lauricola* and of *R. lauricola*-infected host tissue. The SSU primers were used successfully in much of the early work on this disease in Florida, and are still used in experimental work to detect and quantitate *R. lauricola* in artificially inoculated plants (Smith unpublished, Ploetz unpublished). Unfortunately, the SSU primers also amplify DNA from a closely related, previously unknown fungus that was recovered from avocado in 2009, *Raffaelea* sp. 272; it is not pathogenic to avocado and was responsible for the erroneous report mentioned previously (Ploetz and Smith unpublished). Isolates identified with the SSU primers have been tested for pathogenicity on the susceptible avocado cultivar ‘Simmonds’ (Ploetz unpublished) and isolates that cause laurel wilt are presumed to be *R. lauricola*.

Currently, identification of *Raffaelea* spp. relies on ribosomal DNA sequences for the large subunit (LSU) (Harrington et al. 2010). To date, the LSU sequences of each *Raffaelea* spp. has been unique to *Raffaelea* so PCR and DNA sequencing of this region appears reliable and are used to identify unknown isolates as *R. lauricola* (Fraedrich et al. 2008); however, DNA sequencing is more time-consuming than direct PCR diagnosis.

Diagnostic microsatellite sequences of *R. lauricola* were recently identified via 454 pyrosequencing. Their specificity has being tested against related ambrosia fungi (Dreaden and Smith, unpublished). A set of primer pairs for four different microsatellite regions has been selected and is being tested in independent laboratories to ensure its reliability. Use of the vetted technique would enable the rapid and accurate diagnosis of laurel wilt, a necessary first step in managing this disease via sanitation.

V. Response

To date, there are no rules 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 wood from affected trees be burned immediately. Chipping before burning may be helpful, but treatment of such trees with insecticide prior to chipping to avoid disturbance of the vector and encouraging its dispersal to other trees may also be helpful. The latter activities would depend on what is labeled for avocado and effective against *X. glabratus*, as well as whether commercial or residential trees are considered since local burning regulations may vary.

The national clonal germplasm repository for avocado at the USDA-ARS station in Miami, FL maintains about 300 accessions of avocado. In 2009 and 2011, the most important accessions in this collection were protected from laurel wilt by macroinfusion with Alamo (propiconazole) fungicide (see fungicidal management below). In addition, duplication of the most important accessions in this collection began in 2009 by collecting *Avocado sunblotch viroid*–indexed budwood from these accessions and grafting them on seedling rootstocks at the USDA-ARS facility in Ft. Detrick, MD. Eventually, the duplicated germplasm will be transferred to the USDA-ARS facility in Hilo, HI.

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.

Due to the spotty distribution of laurel wilt within the state, experimental work in Florida with *R. lauricola* and *X. glabratus* is conducted under permit from the FDACS.

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 in USDA Hardiness Zones 10-11, with moderate backyard/urban production of some cultivars occurring into Zone 9.

Commercial avocado production is valued at $30 million year^{-1} in Florida and $375 million in California (2003) (Evans 2008; online figures from CA Avocado Commission). In both states, as well as Hawaii and Texas, additional backyard and urban production occurs of unspecified value.

Laurel wilt continues to move south in Florida, but as of April 2011 had not affected commercial avocado production areas. Based on the value of the crop 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).

VIII. Mitigation and Disease Management

Laurel wilt is established so widely in the Southeastern Coastal Plain that eradication of the disease in the USA is not possible. Exclusion of the disease from the remaining unaffected areas

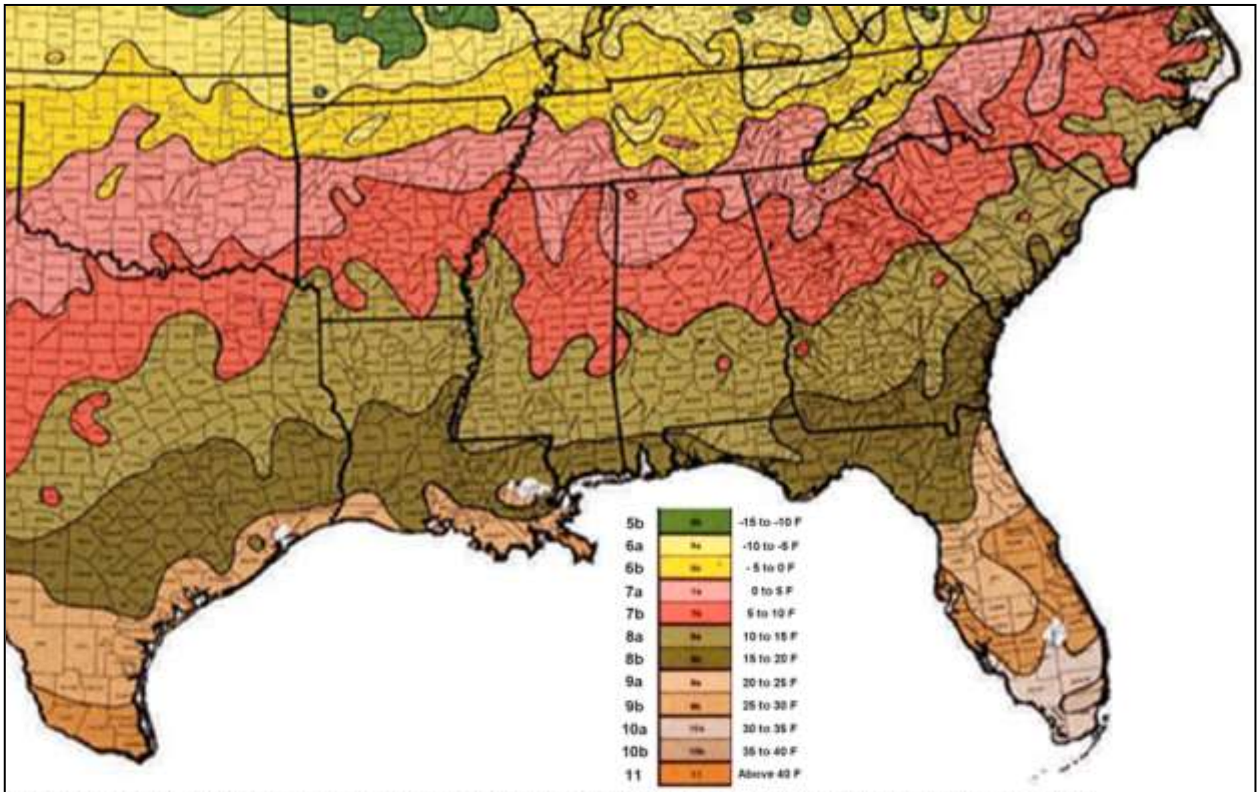


Figure 5. USDA hardiness zones for the southeastern tier of states in the continental USA. Indicated temperatures are the averages winter minimums.

will be difficult. Since movement of infested/affected wood is responsible for long-distance spread of laurel wilt, new firewood regulations in Florida should assist exclusionary efforts in disease-free areas of that state (see: VI. Permit and Regulatory Issues).

Eradication of laurel wilt in a newly affected area has been attempted only once, albeit unsuccessfully (Mayfield et al. 2009). In that situation, only symptomatic redbay trees were removed, and the disease quickly reappeared on the site. Eradication of laurel wilt where suitable brood hosts (e.g., redbay or swampbay) are abundant would be difficult, especially in areas where hosts exist in unmanaged or hard-to-access areas.

Sanitation. The destruction of alternative hosts in and near commercial avocado production areas has been considered. In an extreme suggestion, the creation of a "cordon sanitaire" was proposed (Barnard, personal communication); however, complete or partial removal of alternative hosts in a large area would be difficult, cost-prohibitive and probably environmentally unacceptable. Fraedrich et al. (2011) suggested that laurel wilt in the rare lauraceous hosts (pondberry, *Lindera melissifolia*, and pondspice, *Litsea aestivalis*) could be managed by eliminating redbay within 100 m of conserved stands because the rare hosts are not suitable for brood development of *X. glabratus*.

Where laurel wilt hosts other than avocado are scarce it may be possible to eradicate new outbreaks <u>if</u> affected trees are destroyed before new generations of *X. glabratus* are produced and disperse. The egg-to-mature adult transition has been reported to take 50–60 days for *X. glabratus* in redbay (Hanula et al. 2008, Hanula unpublished data), but shorter periods of 3-4 weeks have been evident in experimental work (Peña unpublished results). The time that elapses between the initiation of external symptoms of laurel wilt and maturation of the first potential vectors in a given tree has not been studied. As this time interval would inform the development of guidelines for disease management via sanitation, some speculation on its duration is warranted.

The first probes into the xylem by *X. glabratus* are thought to inoculate the tree but not result in colonization by and brood establishment of the insect (Fraedrich et al 2008, Harrington and Fraedrich 2010). As symptoms of laurel wilt develop and trees begin to decline, brood galleries are made. With artificial inoculation of avocado seedlings, systemic colonization and external symptoms begin to develop after 12 days, but it takes longer in trees of suitable size for brood development. Thus, a conservative estimate for the symptom-to-vector-dispersal interval might be about 5 weeks (2 weeks for symptom development + 3 weeks for the first life cycle to be completed). Sanitation decisions that would rely on the detection of suspect trees, diagnosis of *R. lauricola*, and subsequent destruction of an infected tree would need to be completed within such a time-frame.

Even if the eradication of new outbreaks in avocado orchards were not possible, sanitation could still play an important role in managing this disease (see: V. Response). Reducing disease pressure via sanitation is an important tactic in managing Dutch elm disease (Stipes 2000).

Resistance. *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 the three races of avocado (Kendra et al. 2011a). 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.

A total of 26 different avocado cultivars have been tested in field trials for response to laurel wilt in Gainesville and Citra, FL since 2007 (Ploetz et al. 2010, Ploetz unpublished). These experiments have utilized grafted trees in 28-60 L pots that were planted in the ground before artificially inoculating with *R. lauricola*. In these tests, WI cultivars have been significantly more susceptible than the G or GxM hybrids (Ploetz et al. 2010). 'Simmonds,' a WI cultivar that comprises 35% of the industry in South Florida, has been consistently among the most susceptible cultivars in this work.

In experiments to assess the impact of plant size on disease development (in redbay, stem diameter is positively correlated with disease development; Fraedrich et al. 2008), disease severity increased significantly on 'Simmonds' as stem diameter increased (Ploetz et al. unpublished). The need for large plants increases the expense of these experiments and restricts testing to those cultivars for which large plants are available. These results also raise concerns about how well the above results reflect what would occur on larger trees.

Ongoing work investigates resistance in the M and G races at Citra, and among open-pollinated seedling progeny from the Miami USDA avocado collection at the USDA station in Ft Pierce, FL. Artificial inoculation of large trees in the field in Miami-Dade County to assess the above concerns regarding the resistance of different cultivars in commercial production situations has not been initiated.

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

In the interest of identifying alternative means to manage the disease, other fungicides and application measures have been evaluated (Ploetz et al. 2011b). Twenty fungicides in 15 chemical groups and 10 fungicide groups were examined in vitro and used to select products for disease suppression on artificially inoculated, potted 'Simmonds.' Soil drench applications of several demethylation inhibitors (DMIs) and thiabendazole provided significant control of the disease ($P<0.05$). The DMI fungicides were equally effective, and since five of these products were triazoles, all members of this chemical group appear 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 used in drench applications of this fungicide. Comparable levels of disease suppression were achieved when propiconazole was applied at 11% of the rate used in soil drenches.

Although topical bark applications would be a less expensive than macroinfusion, moving sufficient concentrations of propiconazole or other fungicides into host xylem will be difficult in trees that are larger than the potted plants that were tested in these trials. Ongoing work examines the means by which this goal might be met on fruit-bearing trees in the field and 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. 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).

Chemical control of the vector is not viewed as a primary management practice but may prove useful in the holistic management of this disease. 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., submitted). 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. 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 baited with Beetle Block (verbenone), a pine bark beetle repellent, had significantly reduced beetle emergence compared to logs that were not baited (Peña et al., submitted). Research is underway to determine the potential of other repellents or protectants, such as methyl jasmonate 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).

Biological control. Biological control measures have been developed for Dutch elm disease (Scheffer et al. 2008). Recently, Shin et al. (2010) reported that an endophytic fungus, *Phaeomoniella* sp., was specifically associated with healthy redbay trees in areas that were affected by laurel wilt. It inhibited growth of *R. lauricola* in vitro, and its potential use as a biocontrol agent against laurel wilt on redbay is being examined.

Predators and parasitoids have been identified on logs infested with *X. glabratus*, but additional research is needed to determine whether any of these species impacts beetle populations (Peña et al. unpublished).

IX. Infrastructure and Experts

Randy Ploetz, a Professor of Plant Pathology at the University of Florida (UF), is an authority on the diagnosis and management of tropical fruit diseases, including those that impact avocado (http://trec.ifas.ufl.edu/personnel_faculty_randy_ploetz.shtml). He has worked on laurel wilt since 2007 and currently researches laurel wilt host responses, resistance and management, and pathogen host range.

The following scientists assisted in the development of this plan.

Pathology, Mycology

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.

Tom Harrington, Professor, Department of Plant Pathology, Iowa State University, Ames, is an authority on insect vectored-pathogens of trees and other associates of bark and ambrosia beetles; senior author of the 2008 publication that described *R. lauricola* as a new fungus; and investigates symbionts of *X. glabratus*.

Sharon Inch, Post-doctoral reseach scientist, University of Florida, investigates the interaction between *R. lauricola* and avocado.

Jason Smith, Assistant Professor of Forest Pathology, School of Forest Resources and Conservation, UF, Gainesville, has experience with laurel wilt on native trees, especially redbay; developed the realtime PCR diagnostic test for *R. lauricola*; and cooperates on avocado resistance screening work with Ploetz.

Mike Wingfield, Mondi Professor of Forest Pathology, FABI and the University of Pretoria, Pretoria, South Africa, is an authority on insect vectored-pathogens of trees and ophiostomatoid fungi.

Entomology, Vector Relations

Jim Hanula, Research Entomologist, Southern Research Station, Forest Service, Athens, GA, conducted the initial work on the biology and ecology of *Xyleborus glabratus.*

Jiri Hulcr, Post-doctoral research scientist, North Carolina State University, Raleigh, NC, Is an expert on ambrosia beetles, their symbionts, and their host interactions.

Paul Kendra, Research Entomologist, USDA-ARS Subtropical Horticultural Research Station, Miami, investigates host attraction for *Xyleborus glabratus.*

Bud Mayfield, Research Entomologist, Forest Service, Asheville, NC, Has considerable experience monitoring the spread of *X. glabratus* and laurel wilt, assessing attractiveness of avocado to *X. glabratus*; and demonstrated fungicide protection of redbay with injected propiconazole.

Jorge Peña, Professor of Entomology, University of Florida, Homestead, was among the first to examine avocado as a host for *X. glabratus* and is currently working on attractants, repellants and insecticides to manage *X. glabratus*.

Bob Rabaglia, Entomologist, Forest Service, Forest Health Protection, Arlington, VA, is a Systematist specializing in the *Xyleborini*. Identified the first USA specimens of *Xyleborus glabratus* and has published the monograph on the genus.

Avocado Germplasm

Raymond Schnell, Research Scientist, USDA-ARS Subtropical Horticultural Research Station, Miami, has extensive experience with the genetic characterization and improvement of avocado; and is responsible for the National Germplasm Repository (NGR) for avocado in Miami (in response to laurel wilt, the NGR collection will be duplicated at the ARS station in Hilo).

Extension

Richard Bostock, Professor and Director, NPDN and WPDN, University of California, Davis, coordinates the diagnostic and education response of the National Plant Diagnostic Network (NPDN) and the Western Plant Diagnostic Network (WPDN) hub of the NPDN.

Jonathan Crane, Professor, Horticultural Sciences, University of Florida, Homestead, Is the Extension specialist with responsibilities for avocado in southern Florida stakeholder/producer interactions and would be involved in detection and first response efforts in Florida.

Akif Eskalen, Cooperative Extension Specialist/Plant Pathologist, Department of Plant Pathology and Microbiology, University of California, Riverside, Is the Extension plant pathologist with statewide responsibilities for avocado and would be involved in detection and first response efforts in California.

Ben Faber, Farm Advisor, University of California Cooperative Extension, Ventura County, is involved with stakeholder/producer interactions and would be involved in detection and first response efforts in California.

Carrie Harmon, University of Florida Co-Director, Southern Plant Diagnostic Network (SPDN), Gainesville, coordinates diagnostic and education responses of the SPDN and would be involved in detection and first response efforts in Florida as well as responsible for compiling Standard Operating Procedures for laurel wilt.

Aaron Palmateer, Assistant Professor and Director, Florida Extension Plant Diagnostic Clinic, University of Florida, Homestead, will be involved in detection and first response efforts in Florida.

Administration

Kent Smith, Plant Pathologist, Office of Pest Management Policy, USDA, Washington, D.C.

X. Research, Extension and Education Priorities

Research. The following laurel wilt research objectives are of primary importance:

- Develop fungicide application measures other than macroinfusion that are effective for administering therapeutic doses of fungicide into the xylem of moderate to large avocado trees (i.e. trees that are most common in commercial production) (Ploetz et al. 2011b). This, work should identify i) fungicides that would result in long-term, efficacious control and ii) cost-effective protocols for application.
- Investigations of insecticides, repellents and attractants that could be used to manage *X. glabratus* in avocado orchards as a component in the management of this disease.
- Genetic resistance to laurel wilt should be sought in existing and related genotypes of avocado.
- Continue to develop accurate and rapid protocols for detecting *R. lauricola* since they are essential for sanitation when managing this disease. To date, good progress has been made with the microsatellite-based approach that is described in IV. Monitoring and Detection, but additional progress will be needed before a widely usable, accurate and rapid protocol is available.
- Important epidemiological data gaps need to be addressed. These include determining: i) whether the pathogen moves via root grafts and pruning equipment; ii) the risk that is posed by the national and international movement of avocado fruit, seed and scions; iii) whether species of ambrosia beetle other than *X. glabratus* are capable of transmitting *R. lauricola* to healthy avocado trees; and iv) the extent to which avocado supports large populations of *X. glabratus* in order to anticipate laurel wilt epidemics prior to their developing in areas where avocado is the sole or primary host tree.

Additional research objectives that are considered of secondary importance include:

- Identifying factors that are associated with or impact the movement of laurel wilt in natural and agroecosystems. For example, relative humidities and temperatures that impact pathogen and vector survival are unknown, as are edaphic, vegetation and other ecological factors.
- Identifying reservoirs of the pathogen and vector among non-hosts (i.e., those that do not develop laurel wilt).

Extension. The University of Florida Extension Service in cooperation with the Florida Department of Plant Industry has provided information on laurel wilt to urban and commercial clientele since 2006. As laurel wilt continues to spread, extending information to diverse clientele will assume even greater importance in Florida, California and other areas in the USA in which avocado is produced and consumed. Current extension efforts that will be important include: in-person and internet-based workshops and presentations, diverse print and online publications, and in-service training of extension faculty.

Education. Several recent epidemics of other tree pests, particularly the emerald ash borer, show that public education and outreach are an indispensable component of comprehensive pest management. Given that the main long-range dispersal route for *X. glabratus* and laurel wilt has been movement of firewood and other forms of infested wood, it is critical that the public be informed about the dangers associated with movement of wood. Although regulations regarding some forms of potentially infested wood are already in place, programs for increasing awareness in the general population are not.

XI. Timeline for Recovery

Recovery within one year of disease onset is not possible with our current capabilities.

Resiliency factors.

- The vector is widespread and continuing to extend its range. At best, our ability to manage the vector is incomplete even in a local sense. Given its ability to successfully transmit the pathogen to avocado with one event, management of laurel wilt via vector control is presently elusive.
- The pathogen in very infective. It can colonize and quickly kill avocados with only one infection event.
- Substantial native reservoirs of the pathogen exist in the affected and threatened avocado-production areas. Based on the available evidence, it appears that they play an important role in pathogen and vector establishment in a given area.

Until effective control measures are identified, it will be difficult to predict how long it will take to recovery from this disease. Successful completion of at least some of the Recommended Next Steps (pg 3) will be needed in order to mitigate this problem, as it is unlikely that sanitation alone will effectively manage the disease.

Based on the prevalence of the crop and presence of a native laurel wilt suscept, California laurel (*Umbellularia californica*), avocado production in California is vulnerable. When and if laurel wilt spreads to this region will depend on the success of firewood interdiction and whether materials infested with *X. glabratus* arrive in western maritime ports. As there are substantial arid areas without significant host populations between the currently infested areas and California, it appears unlikely that natural spread to California would be possible.

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