Review Article

Laurel Wilt in Avocado: Review of an Emerging Disease

Cristina Pisani[1], Randy C. Ploetz[2], Ed Stover[3]*, Mark A. Ritenour[1] and Brian Scully[3]

[1]*Department of Horticultural Sciences, Indian River Research and Education Center, University of Florida, USA*
[2]*Department of Plant Pathology, Tropical Research and Education Center, University of Florida, USA*
[3]*Horticultural Research Laboratory, USDA-ARS, USA*

Abstract

Laurel wilt, caused by the vascular fungus *Raffaelea lauricola*, is transmitted by the red bay ambrosia beetle, *Xyleborus glabratus,* and affects many plants in the family Lauraceae. It was introduced into the United States around 2002 through infested packing material arriving in Georgia. In Florida, the beetle and its associated pathogen have moved progressively throughout the state, arriving in the commercial avocado production area of Miami Dade County in 2011. The fungus grows in galleries and adjacent sapwood of host trees, leading to the disruption of water and nutrient flows. Symptoms include streaks of black discoloration in the sapwood with beetle bore holes on stems and branches of affected trees. Wilt symptoms are associated with the production of gels and tyloses in infected trees. The immediate threat to avocado production in South Florida and the possibility of spread to other states has made identification of control measures a high priority. Current research is testing new fungicides, and different strains of entomopathogenic fungi are showing some efficacy against the red bay ambrosia beetle. However, use of resistant avocado cultivars would likely provide the most sustainable long-term solution. As such, screening of candidate resistant avocado germ plasma by artificial inoculation with the *R. lauricola* pathogen in the field is under way and protocols to facilitate higher throughput screening are also in development. The goal of these efforts is that promising laurel wilt resistant selections with improved horticultural traits and fruit quality for commercial production will be identified.

ABBREVIATIONS

SSU: Small Subunit; LSU: Large Subunit; SSR: Simple Sequence Repeat; SNP: Single Nucleotide Polymorphism; BLAST: Basic Local Alignment Search Tool

INTRODUCTION

The pathogen

Widespread mortality of redbay (*Persea borbonia* (L.) Spreng.) was first observed along the Savannah River and Sea Islands of southeastern South Carolina and around Savannah, GA, USA in 2003. Affected stems and branches presented symptoms including streaks of black discoloration in the sapwood with beetle bore holes on stems and branches of affected trees. Species of ambrosia beetles that were found in symptomatic trees included *Xyleborinus gracilis* Eichhoff and *Ambrosiodmus obliquus* LeConte, which are native to the southeastern USA, and *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), which is native to Southeast Asia [1]. Eventually, *X. glabratus* was shown to transmit a lethal pathogen, the fungus

⬤SciMedCentral

Raffaelea lauricola (Figure 1), which caused laurel wilt and the noted mortality of redbay [1-4].

Laurel wilt is a vascular disease that affects many plants in the family Lauraceae in the USA [2,3]. The fungus grows in galleries and adjacent sapwood of host trees, leading to the disruption of water and nutrient flows. Wilt symptoms are associated with the production of gels and tyloses in infected trees [5]. In the USA, it is most devastating on native hosts such as northern spicebush (*Lindera benzoin* L.), redbay, sassafras (*Sassafras albidum* (Nutt.) Nees), silkbay (*Persea humilis* Nash) and swampbay (*Persea palustris* (Raf.) Sarg.)[1].

Laurel wilt disease has had a deleterious ecological impact in North America, threatening plant communities and fauna associated with members of the Lauraceae family. As the native Lauraceae are lost, the availability of their fruits, nectar, and faunal species that depend on them may be directly and indirectly affected by this disease. For example, larvae of the palamedes swallowtail butterfly, *Papilio Palamedes* Drury, only feed on lauraceous hosts and the spicebush swallowtail, *Papilio troilus* L., use sassafras and northern spicebush as preferred host plants [6]. Laurel wilt may cause changes in habitat by altering light availability, increased woody debris, changes in soil, and other factors. Similar cascades of events have been caused by other exotic tree diseases such as chestnut blight and Dutch elm disease [6]. Furthermore, the distribution of non-native, but economically important species such as camphor tree and avocado may serve as "bridging" or "corridor species" between native host plant habitats, enhancing disease spread to hosts which have discontinuous distributions [6].

R. lauricola first reported on avocado, *Persea americana* Miller, in Jacksonville Florida in September 2007. The tree manifested foliar wilt symptoms (Figure 2) and extensive vascular staining with evidence of burrowing beetle holes. The pathogen was isolated from the discolored sapwood samples and small subunit (18S) sequences from the rDNA were amplified by PCR and sequenced. BLAST nucleotide searches revealed a 100% homology with a *Raffaelea* sp., which was described later as *R. lauricola* [2]. *R. lauricola* is isolated with a semi-selective medium that is used for related anamorphs of the genus *Ophiostoma* and contains cycloheximide, which inhibits the growth of most fungi, but generally not those in the Ophiostomatales [7].

Figure 1 *Raffaelea lauricola* extracted from a cutting of 'Simmonds' avocado through plating onto the selective medium CSMA+.


Figure 2 Foliar symptoms of laurel wilt disease on avocado at the USDA Horticultural Research Laboratory Picos Farm, Ft. Pierce Florida, USA.

The taxonomy of *Raffaelea* spp. is poorly defined [8,9], and a better understanding of the phylogeny of the genus is needed to help diagnosticians, facilitate quarantine efforts, and understand the epidemiology of diseases caused by this phytopathogen. Most *Raffaelea* spp. live as saprophytes; but, *R. lauricola*, *R. quercivora*, and *R. quercus-mongolicae* affect economically and ecologically important members of the family Lauraceae (laurel wilt) and *Quercus* spp. (Japanese and Korean oak wilt) [10]. Laurel wilt disease is now established in the southeastern United States and has the potential to reach the U.S. Pacific coast, Mexico, and Central and South America, threatening the avocado industry in those areas. Studies have shown that the California bay laurel (*Umbellularia californica* Hook. & Arn.(Nutt.)) as well as other Lauraceous hosts are susceptible to laurel wilt, hence posing a major threat to the Pacific coastal ecosystem [4,6]. Public awareness is needed on the risks of transport and movement of infested wood as well as the implementation of adequate quarantine protocols.

Multigene genealogies have demonstrated that *Raffaelea* is polyphyletic, and that the currently described species fall in two clades [8,9]. The *Ophiostoma* clade includes *R. lauricola*, *R. brunnea*, and one undescribed species of the genus from Canada, while *Raffaelea* spp. such as *R. quercivora*, *R. montetyi*, *R. sulphurea*, and *R. amasae* fall into the *Leptographium* clade [8,9]. There are still undescribed *Raffaelea* taxa that need to be properly classified.

PCR amplification of small subunit (SSU; 18S) or large subunit (LSU; 28S) sequences from the rDNA have been used to detect *R. lauricola* and diagnose laurel wilt [2,11,12]. However, there are pitfalls when pathogens reside in poorly defined genera such as *Raffaelea*. In one case, the SSU method gave a false positive for a dead avocado tree that was examined in 2009, identifying an isolate, PL1004, as *R. lauricola*. PL1004 was later shown to be non-pathogenic on avocado and was recently shown to be a new species [8,9]. A detection method developed by Jeyaprakash et al. [12], which uses a section of the LSU, also fails to distinguish PL1004 from *R. lauricola*. Although the SSU and LSU sequences are not *R. lauricola*-specific and, thus, cannot be used conclusively for diagnostic purposes, the SSU amplicon is useful in experimental situations (e.g. after artificial inoculations)

SciMedCentral
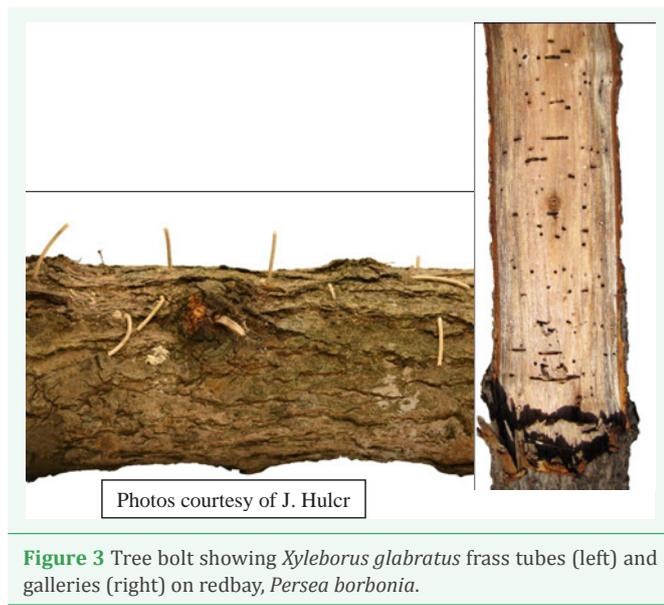
Figure 3 Tree bolt showing *Xyleborus glabratus* frass tubes (left) and galleries (right) on redbay, *Persea borbonia*.

since it can detect ca. 0.0001 ng of the pathogen DNA. Recently, Dreaden et al. [8] developed two small sequence repeat (SSR or microsatellite) markers for *R. lauricola* (microsatellites in fungi have limited intertaxon transferability, vary in length and exhibit less polymorphism than in other organisms [13,14]). The SSR markers of Dreaden et al. [8] are *R. lauricola*-specific and can be used to identify the pathogen in culture, but have a detection limit of only 0.1 ng of pathogen DNA. Four labs tested and confirmed the consistency of the methodology, which is now currently used to diagnose laurel wilt/identify *R. lauricola* [8]. In summary, the SSU markers are not taxon specific, but are highly sensitive, whereas the microsatellite markers are unable to detect low titers of the pathogen, but are taxon-specific and both are used in the diagnosis of the pathogen.

Host colonization and disease development has been studied in avocado, swampbay, and camphor tree with a GFP-labeled strain of *R. lauricola* [15]. Little colonization was observed, even in severely affected trees (<1.6%). Nonetheless, resistance to laurel wilt was associated with lower levels of xylem colonization, assayed as the percentage of colonized xylem in a given cross-section of the stem [15].

R. lauricola is a clonal pathogen in the USA (putative founder effect of a single strain) [16]. Isolates from the USA share strong homology with strains from Taiwan and Japan, thus supporting the hypothesis that *R. lauricola* in the USA originated from Asia [11]. Likewise, *R. fusca* and *R. subfusca* were also isolated from *X. glabratus* from Taiwan, suggesting that these *Raffaelea* species were also introduced into the USA from Asia [11].

Vector(s)

In the order Coleoptera, there are 90 families in the infraorder Curcujiformia of which Curculionidae contains the subfamilies Platypodinae and Scolytinae that include the bark and ambrosia beetles. There are about 3,400 known species of ambrosia beetles [10]. Bark beetles colonize phloem, but ambrosia beetles have evolved to colonize the xylem tissue of woody plants a they have symbiotic relationships with fungi. Available evidence indicates that the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) in the tribe Xyleborini, was introduced with its fungal symbiont, *R. lauricola*, into Georgia around 2002 in infested packing material, such as wooden crates or pallets [17,18]. It has since spread north in southeastern Virginia in 2011 and west into a few counties of Mississippi and Louisiana in 2009, 2013, and 2014. During the spring of 2005, the beetle was detected in Duval County, Florida and has since spread along the east coast of Florida as far south as Indian River County in 2006, Brevard County in 2007, Okeechobee and Osceola counties in 2008, and Miami Dade County in 2011 [19].

Females of *X. glabratus* and of other ambrosia beetles carry fungal spores in specialized mandibular structures called mycangia. Males are haploid, smaller than females and cannot fly, whereas females are diploid, strong fliers, and are responsible for dispersion of *R. lauricola* [1,3]. Females bore into the xylem of host trees to create galleries that they inoculate with *R. lauricola*; adults and larvae then feed on *R. lauricola* that proliferates in galleries (Figure 3). *X. glabratus* has haplo-diploid sex determination and females are able to reproduce without mating. This allows isolated females to locate a new host and lay unfertilized eggs that will give rise to males. The parental female can then mate and produce diploid eggs that will hatch into females and able to disperse to colonize new hosts [4]. The fungal diet of *X. glabratus* enables it to colonize the xylem of host trees, which is nutrient-poor but provides a protected habitat for brood development. Studies have shown that flight patterns are specie: specific for the Scolytinae [20,21]. *Xyleborus* spp. generally initiates flight at about one hour before sunset. However, *X. glabratus* was observed to start flight a few hours earlier, which may indicate the use of visual cues for host location [21]. This can help improve its detection and develop attractant traps for pest control. Studies show that Scolytinae beetles fly close to the ground with the highest number of *X. glabratus* captured at 35-100 cm above the ground [20]. Trap captures in Florida revealed peak activities for *X. glabratus* in September-October and February-April. Small peaks were observed in May-August and December-January which may be attributed to age of the manuka oil lure that is known to last only two weeks, as well as rainfall, and lower temperatures in the winter [20].

The ecological niche of ambrosia beetles is typically stressed, dying or dead trees in which the insects can propagate. Thus, interactions of ambrosia beetles with "healthy" trees have been viewed as atypical and a probable indication that the host trees were stressed by drought, flooding, freeze damage, wind damage, and poor cultural practices [22], or biotic stresses such as Phytophthora root rot (cause by *P. cinnamomi*) [23,24].

As laurel wilt spread in the USA, it was noted that *X. glabratus* attacked both healthy trees and those that were already affected by laurel wilt disease. To explain the supposed atypical interaction of *X. glabratus* with healthy hosts, several hypotheses were proposed. Hulcr and Dunn [25] suggested that an "olfactory mismatch" occurred in which plant volatiles that are specific to stressed trees in the native habitat of *X. glabratus* were produced by healthy trees in the new regions. A "permissive choice hypothesis" proposed that selection in the beetle's native environment ensured that they were attracted to stressed or

dying trees rather than healthy trees with greater natural plant defenses [25]. However, in new ranges or habitats natural selection of beetles resulted in beetles that were attracted to healthy trees, enabling them to avoid competition with native beetles. An alternative explanation for these relationships may be that selection pressure for susceptibility in the home range of *R. lauricola* culled all hosts except those that had useful resistance. Ploetz et al. [10] suggested that ambrosia beetles as a group may actually interact with healthy trees more often than the above hypotheses suggest. They noted that the only reason *X. glabratus* was known to interact with healthy trees was due to its dissemination of a lethal pathogen. Experimental results for other ambrosia beetle species transmitting *R. lauricola* were published recently [26]. Clearly, better understandings are needed for how and when ambrosia beetles interact with healthy trees.

Studies on plant and fungal volatiles have sought to identify attractants to help deter or capture the redbay ambrosia beetle [27-30]. Phoebe oil lures were very efficient in attracting *X. glabratus*, but are no longer available due to overharvesting and the scarcity of phoebe oil trees in their natural habitat in Brazil [31]. Manuka oil lures are used instead, but research shows that these lures only last up to two weeks in the field [32]. Evaluation of seven essential oils has shown that the greatest number of redbay ambrosia beetles was captured using cubeb, manuka, and phoebe oils and that the addition of ethanol as a potential synergist had no effect on the number of beetles captured [32]. Studies have shown that plant volatiles such as α-copaene, β-caryophyllene, and α-humulene have been positively correlated with field captures of *X. glabratus.* Most recently, four sesquiterpenes were confirmed to attract beetles: α-copaene, α-humulene, α-cubebene, and calamenene. Alpha-cubebene and α-copaene are the two major components in cubeb oil and were found to be the major attractants in susceptible hosts with α-cubebene being the stronger attractant of the two. Further studies confirm that cubeb lures are currently the best attractants for *X. glabratus* detection and can last for at least eight weeks in the field [27-29]. It is likely that females are attracted to multiple volatile compounds emitted by the Lauraceae, not a single kairomone. However, this research did not examine the female beetles' specific preferences among the three known horticultural races of avocado (races discussed below), despite the different chemical profiles produced by each avocado race as determined by gas chromatography-mass spectrometry (GC-MS) analysis [27]. Beetles have shown a preferential attraction to lychee, *Litchi chinensis* Sonnerat, which is not susceptible to laurel wilt, but more females bore into avocado wood over a longer period of time [28]. This suggests that beetles are initially more strongly attracted to lychee wood volatiles, but find it to be an unsatisfactory substrate to grow their fungal symbiont and hence relocate to look for a more suitable long-term host [27]. Ambrosia beetles are also attracted to volatiles produced by their corresponding fungal symbionts, which may enable their orientation within a gallery or location of established fungal gardens of conspecific beetles [30]. These findings may ultimately help engineer species-specific lures for beetle and disease control.

There is usually a high level of specificity between ambrosia beetles and their fungal symbionts. Recently, the lateral transfer of *R. lauricola* to, and its dissemination by, six other ambrosia beetle

species was reported [26]. *Xyleborus affinis*, *X. ferrugineus*, *X. volvulus* and *Xyleborinus gracilis* are endemic to tropical America and the southeastern USA, whereas *Xylosandrus crassiusculus* Motschulsky and *Xyleborinus saxeseni* Ratzeburg are non-native beetles that have established in the USA. The capacity of several beetle species to carry this pathogen could conceivably enable an expanded host range for laurel wilt, as the other species have wider host ranges than *X. glabratus*. More importantly, the ability of these beetles to transmit *R. lauricola* to avocado and/or redbay [26], and the current absence of *X. glabratus* in laurel wilt-affected avocado groves in Miami-Dade County [Carrillo et al., unpublished data] indicate that the other species may play a role in the epidemiology of this disease on this crop.

Host trees

To date, laurel wilt disease has not been reported in its the native Asian countries (Taiwan, Japan, India, and Myanmar) of *R. lauricola* and *X. glabratus* [11]. It has been hypothesized that Asian members of the family coevolved with the pathogen, resulting in varying levels of resistance, while North American hosts have not [10]. For example, the camphor tree (*Cinnamomum camphora* L.) is a Lauraceae species of Asian origin that typically recovers after infection [33,34]. Although trees may have coevolved an accommodating response to these fungi in Asia, the host features that are responsible for susceptibility in some trees in the Western hemisphere are not clear. Nonetheless, avocado does respond rapidly to artificial inoculation with *R. lauricola.* Tyloses and gels were induced in the xylem shortly after inoculation [35], which was rapidly colonized by the pathogen [23]. Yet, there was surprisingly little histological evidence of the pathogen in these trees. Xylem function and xylem conductivity were significantly correlated and reduced dramatically after infection [5,35]. Trees with a larger stem diameter develop more severe and rapid wilting symptoms compared with smaller diameter trees [23]. Other studies indicate that as few as 100 conidia can kill an entire tree [36]. More information is needed on the features of resistant host species.

The host range of redbay is throughout the coastal plains of the southeastern United States extending along the Atlantic coast from southern Virginia to southern Florida and west along the Gulf of Mexico to eastern Texas. The highest concentrations of redbay are found in southern Georgia and the Albemarle Peninsula of eastern North Carolina [37]. Other Lauraceous hosts such as sassafras have much wider host ranges, with low densities in the southeastern United States, Michigan, and north into New Hampshire. Highest concentrations of sassafras in eastern Oklahoma to Ohio and West Virginia [37]. The broad range of redbay and other native host trees throughout Florida facilitated the spread of *R. lauricola* and *X. glabratus* to the commercial avocado production area in Miami-Dade County, where it was detected in early 2011 [4,10]. Now that it is established in South Florida, laurel wilt is a major threat to commercial avocado production and the valuable collections in the USDA-ARS national avocado germplasm repository in Miami. Cuttings of the collection are currently in quarantine in Beltsville, Maryland so that is can be moved to a safe location in Hawaii. Avocado is a high-value specialty crop in the US, grown commercially primarily in California and Florida. The Florida avocado industry

Stover et al. (2015)
Email: Ed.Stover@ARS.USDA.GOV

SciMedCentral

contributes nearly $30 million to the local economy, with production of 31,100 tons and with more than 6,773 production acres in Miami-Dade County alone. A production and marketing report illustrated how direct cost estimates are broken down into potential sale losses, decreased property values, and increased management costs as a consequence of laurel wilt disease. It suggests the entire industry could be destroyed and agricultural property values could lose one-half of their market value if the disease continues to spread [38]. As a consequence, avocado imports and/or domestic production by other avocado-producing states would need to increase to meet current demand in the United States. California is the major producer of avocado in the United States with a production of 195,000 tons in 2012 (NASS). It was confirmed that *R. lauricola* can cause disease on California laurel *Umbellularia californica* (Hook. &Arn.) Nutt. and bay laurel *Laurus nobilis* L., which could help spread the disease within California if introduced into that state [6]. Other avocado-producing countries such as Mexico and Chile could have devastating losses from laurel wilt disease. The immediate threat in South Florida and the possibility of spread to other states has made identification and implementation of control measures a high priority.

Avocado is an evergreen subtropical fruit tree of neotropical origin in the family Lauraceae. This species is characterized by three botanical races that originated in Guatemala, Mexico and Central America [39,40]. Scion cultivars used in California include Bacon, Gwen, Reed, Zutano, Pinkerton, Hass, and Fuerte, all of which are derived from the Mexican and/or Guatemalan races. In Florida, commercial cultivars are all Antillean (i.e.,West Indian) or Antillean x Guatemalan hybrids such as Lula, Booth 8, Waldin, Simmonds, Donnie and Choquette [40,41]. The three avocado races are easily distinguished from each other in that Mexican types are semi-tropical, usually more tolerant to colder environments and are smaller trees with anise-scented leaves. Fruits of Mexican cultivars have thin, smooth, and dark skin and take up to 6 months to reach maturity. Guatemalan varieties are subtropical, intermediate in cold tolerance and are able to grow at high attitudes (900 m to 2,400 m). In contrast, Antillean cultivars prefer tropical environments and generally are more cold sensitive. Ploetz et al. [23] reported differences in the severity of laurel wilt disease development across different avocado cultivars, with Antillean cultivars being more susceptible than those with Mexican or Guatemalan backgrounds.

On avocado, external laurel wilt symptoms appear as wilting of the terminal leaves that rapidly change color from dark green to brown right after wilting occurs (Figure 2). Unlike redbay, in which leaves do not detach from the tree for a year or longer, avocado can defoliate in as little as 2 to 3 months from the first symptom development [42]. This may be due to the higher susceptibility of redbay that leads to a faster disease development and inadequate time for leaf abscission zones to develop. Internal symptoms develop faster than external symptoms and when wilting of leaves is observed internal symptoms are advanced. Sapwood turns to a reddish brown to blue-grey with streaks [23], resembling symptoms of Dutch elm disease.

Management

Currently, laurel wilt challenges the Florida industry and threatens those in Texas, California, Mexico and the Caribbean. The following integrated pest management program has been recommended wherein early detection is based on growers' visual scouting: suspect wood samples are collected and taken to county and university diagnostic labs for confirmation of *R. lauricola*; positive trees are then promptly removed, chipped and sprayed with insecticide containing permethrin (Permethrin 3.2 AG; Arysta Life Science North America, Cary NC or Permethrin 3.2 EC; Helena Chemical, Collierville, TN) [38]; and adjacent, surrounding "healthy" trees are treated with Tilt, propiconazole (Syngenta Crop Protection LLC, Greensboro, NC, USA) to impede root graft transmission of the pathogen (primary means of disease spread in affected orchards) (Ploetz, personal communication). Wood that is too large to chip should be burned. Studies show that disease development was prevented when Tilt fungicide treatment occurred before inoculation compared to treatment after inoculation [42]. This may be due to the fact that tyloses may prevent systemic movement of the fungicide up the xylem vessels. The fungicide is delivered via macro-infusion, which is a most effective application measure but is slow and expensive and not commercially viable for treating entire groves. Current research is being conducted on the effectiveness and residual lifespan of additional fungicides within the tree. Although thiabendazole has been shown to have a longer lifespan than propiconazole and is currently being used to protect against Dutch elm disease, it is ineffective against laurel wilt [42]. Studies to develop effective delivery methods for Propiconazole Pro, tebuconazole, and Tilt are underway (Ploetz, unpublished). Unfortunately, it is impractical to use quarantine practices to limit spread of the beetle as it is already well established along the Southeastern United States. It is however strongly advised to not move or sell redbay and other host trees as firewood to minimize the spread of the beetle and pathogen to unaffected areas.

Extensive laurel wilt infection of native redbay and swampbay in Merritt Island, FL has been a concern for the small avocado groves in the area, which is far north of the primary avocado production area. It was observed that avocado groves in this area were only randomly attacked compared to redbay stands over a 3-4 year period. However, this could change once the redbay population declines and beetles scout for alternative hosts. Another explanation could be that *X. glabratus* may play a much more limited role in the spread of laurel wilt to avocado than originally thought and alternative vectors needed for transmission to avocado are not present in the area [43].

Recently, entomopathogenic fungi were tested against the redbay ambrosia beetle. Two commercial strains of *Isaria fumosorosea* and one strain of *Beauveria bassiana* were found to effectively kill female beetles in galleries, thus preventing beetle reproduction and suppressing the establishment of their fungal symbiont in galleries [44]. It was shown that median survivorship times of female beetles ranged from as little as 3 days for *B. bassiana* to 5 days for *I. fumosorosea* strains. These biocontrol strains were not tested for effects on *R. lauricola* establishment and the potential for disease transmission via root graft needs to also be considered.

Vector and inoculum management strategies as well as fungicide applications are critical to protect established plantings,

Stover et al. (2015)
Email: Ed.Stover@ARS.USDA.GOV

ⒸSciMedCentral

but use of resistant avocado cultivars would provide the most sustainable long-term solution. Screening of the germplasm by artificial inoculation of the *R. lauricola* pathogen will aid in identifying tolerance or resistance.

Seedlings from diverse avocado parents are being subjected to field assessments (Pisani et al., unpublished). Protocols to facilitate higher throughput screening are also in development. Ideally, once truly resistant materials are available, Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers could help identify tolerance or resistance genes to the disease more efficiently and accurately. It is hoped that promising laurel wilt resistant selections with desirable horticultural traits and resistance to priority diseases for commercial production will be identified.

Since avocado consumption is increasing and a large proportion of avocados eaten in the United States are currently imported, expanded U.S. avocado production has significant potential. However, laurel wilt resistance will be a key factor in the sustainability of this crop. Without prompt identification and disposal of affected trees and fungicide treatment of vulnerable adjacent trees, it is currently impossible to impede the disease's spread in affected orchards [4]. Substantial research is needed on the disease's epidemiology and improved measures for its management.

REFERENCES

1. Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield III AE, Hanula JL, et al. A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other lauraceae in the southeastern united states. Plant Dis. 2008; 92: 215-224.

2. Mayfield III AE, Smith JA, Hughes M, Dreaden TJ. First report of laurel wilt disease caused by raffaelea sp. On avocado in florida. Plant Dis. 2008; 92: 976.

3. Hanula JL, Mayfield AE 3rd, Fraedrich SW, Rabaglia RJ. Biology and host associations of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae), exotic vector of laurel wilt killing redbay trees in the southeastern United States. J Econ Entomol. 2008; 101: 1276-1286.

4. Kendra PE, Montgomery WS, Niogret J, Epsky ND. An uncertain future for American Lauraceae: a lethal threat from redbay ambrosia beetle and laurel wilt disease (a review). Am J Plant Sci. 2013; 4: 727-738.

5. Inch S, Ploetz RC. Impact of laurel wilt, caused by Raffaelea lauricola, on xylem function in avocado, Persea americana. Forest Pathol. 2012; 42: 239-245.

6. Gramling LM. Potential effects of laurel wilt on the flora of North America. Southeast Nat. 2010; 9: 827-836.

7. Harrington TC. Cycloheximide sensitivity as a taxonomic character in Ceratocystis. Mycologia. 1981; 73: 1123-1129.

8. Dreaden TJ, Davis JM, Harmon CL, Ploetz RC, Palmateer AJ, Soltis PS, et.al. Development of multilocus PCR assays for Raffaelea lauricola, causal agent of laurel wilt disease. Plant Dis. 2014; 98: 379-383.

9. Dreaden TJ, Davis JM, de Beer ZW, Ploetz RC, Soltis PS, Wingfield MJ, et al. Phylogeny of ambrosia beetle symbionts in the genus Raffaelea. Fungal Biol. 2014; 118: 970-978.

10. Ploetz RC, Hulcr J, Wingfield MJ, de Beer ZW. Destructive tree diseases associated with ambrosia and bark beetles: Black swan events in tree pathology? Plant Dis. 2013; 97: 856-872.

11. Harrington TC, Yun HY, Lu S-S, Goto H, Aghayeva DN, Fraedrich SW. Isolations from the redbay ambrosia beetles, Xyleborus glabratus, confirm that the laurel wilt pathogen, Raffaelea lauricola, originated in Asia. Mycologia. 2011; 103: 1028-1036.

12. Jeyaprakash A, Davison DA, Schubert TS. Molecular detection of the laurel wilt fungus, Raffaelea lauricola. Plant Dis. 2014; 98: 559-564.

13. Dutech C, Enjalbert J, Fournier E, Delmotte F, Barrès B, Carlier J, et al. Challenges of microsatellite isolation in fungi. Fungal Genet Biol. 2007; 44: 933-949.

14. Cristancho M, Escobar C. Transferability of SSR markers from related Uredinales species to the coffee rust Hemileia vastatrix. Genet Mol Res. 2008; 7: 1186-1192.

15. Campbell AF. Dissertation. Gainesville (FL): University of Florida, 2014.

16. Hughes M. Dissertation. Gainesville (FL): University of Florida, 2014.

17. Thomas MC. Two Asian ambrosia beetles recently established in Florida (Curculionidae: Scolytinae). Dept. Plant Industry, Florida Department of Agriculture and Consumer Services. 2007.

18. Rabaglia RJ, Dole SA, Cognato AI. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. Ann Entomol Soc Am. 2006; 99: 1034-1056.

19. Ploetz RC, Peña JE, Smith JA, Dreaden TL, Crane JH, Schubert T, et.al. Laurel wilt, caused by Raffaelea lauricola, is confirmed in Miami-Dade County, center of Florida's commercial avocado production. Plant Dis. 2011. 95:1589.

20. Brar GS, Capinera JL, McLean S, Kendra PE, Ploetz RC, Peña JE. Effect of trap size, trap height and age of lure on sampling Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae), and its flight periodicity and seasonality. Fla Entomol. 2012; 95: 1003-1011.

21. Kendra PE, Montgomery WS, Niogret J, Deyrup MA, Guillén L, Epsky ND. Xyleborus glabratus, X. affinis, and X. ferrugineus (Coleoptera: Curculionidae: Scolytinae): Electroantennogram responses to host-based attractants and temporal patterns in host-seeking flight. Chem Ecol. 2012; 41: 1597-1605.

22. Ranger CM, Reding ME, Persad AB, Herms DA. Ability of stress-related volatiles to attract and induce attacks by Xylosandrus germanus and other ambrosia beetles. Agric Forest Entomol. 2010; 12: 177-185.

23. Ploetz RC, Perez-Martinez JM, Smith JA, Hughes M, Dreaden TJ, Inch SA, et al. Responses of avocado to laurel wilt, caused by Raffaelea lauricola. Plant Pathol. 2012; 61: 801-808.

24. Dann EK, Ploetz RC, Coates LM, Pegg KG. Foliar, fruit, and soilborne diseases. The avocado: botany, production and uses. 2013; 380-415.

25. Hulcr J, Dunn RR. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. Proc Biol Sci. 2011; 278: 2866-2873.

26. Carrillo D, Duncan RE, Ploetz JN, Campbell AF, Ploetz RC, Peña JE. Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathol. 2014; 63: 54-62.

27. Kendra PE, Montgomery WS, Niogret J, Peña JE, Capinera JL, Brar G, Epsky ND. Attraction of the redbay ambrosia beetle, Xyleborus glabratus, to avocado, lychee, and essential oil Lures. J Chem Ecol. 2011; 37: 932-942.

28. Kendra PE, Ploetz RC, Montgomery WS, Niogret J, Peña JE, Brar GS, et al. Evaluation of Litchi chinensisfor host status to Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae) and susceptibility to laurel wilt disease. Fla Entomol. 2013; 96(4): 1442-1453.

29. Kendra PE, Montgomery WS, Niogret J, Pruett GE, Mayfield AE III, MacKenzie M, et.al. North American Lauraceae: Terpenoid emissions,

relative attraction and boring preferences of redbay ambrosia beetle, Xyleborus glabratus (c). PLoS ONE. 2014; 9: e102086.

30. Hulcr J, Mann R, Stelinski LL. The scent of a partner: ambrosia beetles are attracted to volatiles from their fungal symbionts. J Chem Ecol. 2011; 37: 1374-1377.

31. Hanula JL, Sullivan BT, Wakarchuk D. Variation in manuka oil lure efficacy for capturing Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae), and cubeb oil as an alternative attractant. Chem Ecol. 2013; 42: 333-340.

32. Kendra PE, Montgomery WS, Niogret J, Schnell EQ, Deyrup MA, Epsky ND. Evaluation of seven essential oils identifies cubeb oil as most effective attractant for detection of Xyleborus glabratus. J Pest Sci. 2014; 87: 681-689.

33. Fraedrich SW, Harrington TC, Best GS. Xyleborus glabratus attacks and systemic colonization by Raffaelea lauricola associated with dieback of Cinnamomum camphora in the southeastern United States. For Path. 2015; 45: 60-70.

34. Smith JA, Mount L, Mayfield AE III, Bates CA, Lamborn WA, Fraedrich SW. First report of laurel wilts disease caused by Raffaelea lauricola on camphor in Florida and Georgia. Plant Dis. 2009; 93:198.

35. Inch S, Ploetz R, Held B, Blanchette R. Histological and anatomical responses in avocado, Persea americana, induced by the vascular wilt pathogen, Raffaelea lauricola. Botany. 2012; 90: 627-635.

36. Hughes MA, Inch SA, Ploetz RC, Er HL, van Bruggen AHC, Smith JA. Responses of swamp bay, Persea palustris, and avocado, Persea americana, to various concentrations of the laurel wilt pathogen, Raffaelea lauricola. For Path. 2015; 45: 111-119.

37. Koch FH, Smith WD. Spatio-temporal analysis of Xyleborus glabratus (Coleoptera: Circulionidae: Scolytinae) invasion in Eastern U.S. forests. Environ Entomol. 2008; 37: 442-452.

38. Evans EA, Crane J, Hodges A, Osborne JL. Potential economic impact of laurel wilt disease on the Florida avocado industry. Hort Technology. 2010; 20: 234-238.

39. Schaffer, BA, Wolstenholme BN, Whiley AW. The avocado: botany, production and uses. 2nd Edn. CABI. 2013.

40. Litz RE, Witjaksono, Raharjo S, Efendi D, Pliego-Alfaro F, Barceló-Muñoz A. 11.1 *Persea americana* Avocado. Biotechnology of fruit and nut crops. CABI 2005; 326-347.

41. Ploetz RC, Zentmyer GA, Nishijima WT, Rohrbach KG, Ohr HD. Compendium of Tropical Fruit Diseases. The American Phytopathological Society. 1994.

42. Ploetz RC, Pérez-Martínez JM, Evans EA, Inch SA. Toward fungicidal management of laurel wilt of avocado. Plant Dis. 2011; 95: 977-982.

43. Crane J, Peña JE, Ploetz RC, Smith J, Evans EA. Proposed strategies for decreasing the threat of laurel wilt (LW) and its vector, the redbay ambrosia beetle (RAB) to commercial avocado groves in Miami-Dade County. IFAS. 2011.

44. Carrillo D, Dunlap CA, Avery PB, Navarrete J, Duncan RE, Jackson MA, et.al. Entomopathogenic fungi as biological control agents for the vector of laurel wilt disease, the redbay ambrosia beetle, Xyleborus glabratus (Coleoptera: Curculionidae). Biol Control. 2015; 81: 44-55.