Laurel Wilt in Avocado: Review of an Emerging Disease

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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 Raffaelea lauricola.
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 (Raef) 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 Lauraceae 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, other exotic tree diseases such as chestnut blight and Dutch elm disease [6].

*Raffaelea 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 *Raffaelea* sp., which was described later as *R. lauricola* [2]. *R. lauricola* is isolated with a semi-selective medium such as *R. brunnea*, and one undescribed species of the genus from Canada, while *Raffaelea* spp. such as *R. quercivora*, *R. montetyi*, and *R. amasae* fall into the *Leptographium* clade [8,9]. There are still undescribed *Raffaelea* taxa that need to be properly classified.

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* [9]. 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).
Host colonization and disease development has been studied in avocado, swampbay, and camphor tree with a GFP-labeled pathogen, but are taxon-specific and both are used in the diagnosis of the pathogen. Studies have shown that flight patterns are species 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 ambrosia beetles. An alternative explanation for these relationships may be that beetles are initially more strongly attracted to lychee wood over a longer period of time [28]. This suggests 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]. *Xyloborus affinis*, *X. ferrugineus*, *X. volvulus* and *Xyleborinus gracilis* are endemic to tropical America and the southeastern USA, whereas *Xylosandrus crassiusculus* Motschulsky and *Xyloborinus 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 xylems. 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
Central Sapwood turns to a reddish brown to blue-grey with streaks [23], symptoms develop faster than external symptoms and when and inadequate time for leaf abscission zones to develop. Internal susceptibility of redbay that leads to a faster disease development symptom development [42]. This may be due to the higher avocado can defoliate in as little as 2 to 3 months from the first which leaves do not detach from the tree for a year or longer, to brown right after wilting occurs (Figure 2). Unlike redbay, in those with Mexican or Guatemalan backgrounds. cultivars, with Antillean cultivars being more susceptible than 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 altitudes (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 lead 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 aditonal fungicides within the tree. Although thiabendazole has 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,
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 proportion of avocados eaten in the United States are currently imported, and lauricola, to avocado, lychee, and essential oil Lures. J Chem Ecol. 2012; 41: 1597-1605.


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