

Article

Laurel Wilt in Natural and Agricultural Ecosystems: Understanding the Drivers and Scales of Complex Pathosystems

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Abstract: Laurel wilt kills members of the Lauraceae plant family in the southeastern United States. It is caused by *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, a nutritional fungal symbiont of an invasive Asian ambrosia beetle, *Xyleborus glabratus* Eichhoff, which was detected in Port Wentworth, Georgia, in 2002. The beetle is the primary vector of *R. lauricola* in forests along the southeastern coastal plain of the United States, but other ambrosia beetle species that obtained the pathogen after the initial introduction may play a role in the avocado (*Persea americana* Miller) pathosystem. Susceptible taxa are naïve (new-encounter) hosts that originated outside Asia. In the southeastern United States, over 300 million trees of redbay (*P. borbonia* (L.) Spreng.) have been lost, and other North American endemics, non-Asian ornamentals and avocado—an important crop that originated in MesoAmerica—are also affected. However, there are no reports of laurel wilt on the significant number of lauraceous endemics that occur in the Asian homeland of *R. lauricola* and *X. glabratus*; coevolved resistance to the disease in the region has been hypothesized. The rapid spread of laurel wilt in the United States is due to an efficient vector, *X. glabratus*, and the movement of wood infested with the insect and pathogen. These factors, the absence of fully resistant genotypes, and the paucity of effective control measures severely constrain the disease’s management in forest ecosystems and avocado production areas.

Keywords: laurel wilt; Lauraceae; redbay; avocado; *Raffaelea lauricola*; *Xyleborus glabratus*; ambrosia beetles; coevolution

1. Introduction

New diseases are developing at an alarming rate on the world’s trees. Diverse forest communities, pulp and timber plantations, and agricultural production are impacted in tropical, temperate and boreal environments [1–4]. Host jumps, pathogen hybridization and climate change have been associated with some of the new diseases [5–8], but other outbreaks have resulted from the invasion of naïve

ecosystems by exotic pathogens [9–12]. The occasionally catastrophic responses of new-encounter hosts to alien pathogens are poorly understood, unpredictable and warrant further study [1].

In 2004, stands of redbay, *Persea borbonia* (L.) Spreng., began to die on Hilton Head Island, South Carolina, United States [13] (Figure 1A). At the time, the problem was not widespread, and abiotic factors, such as drought or salt-water damage, were invoked as possible causes for losses of this native tree. However, as the problem spread a new disease, laurel wilt, was recognized [13].

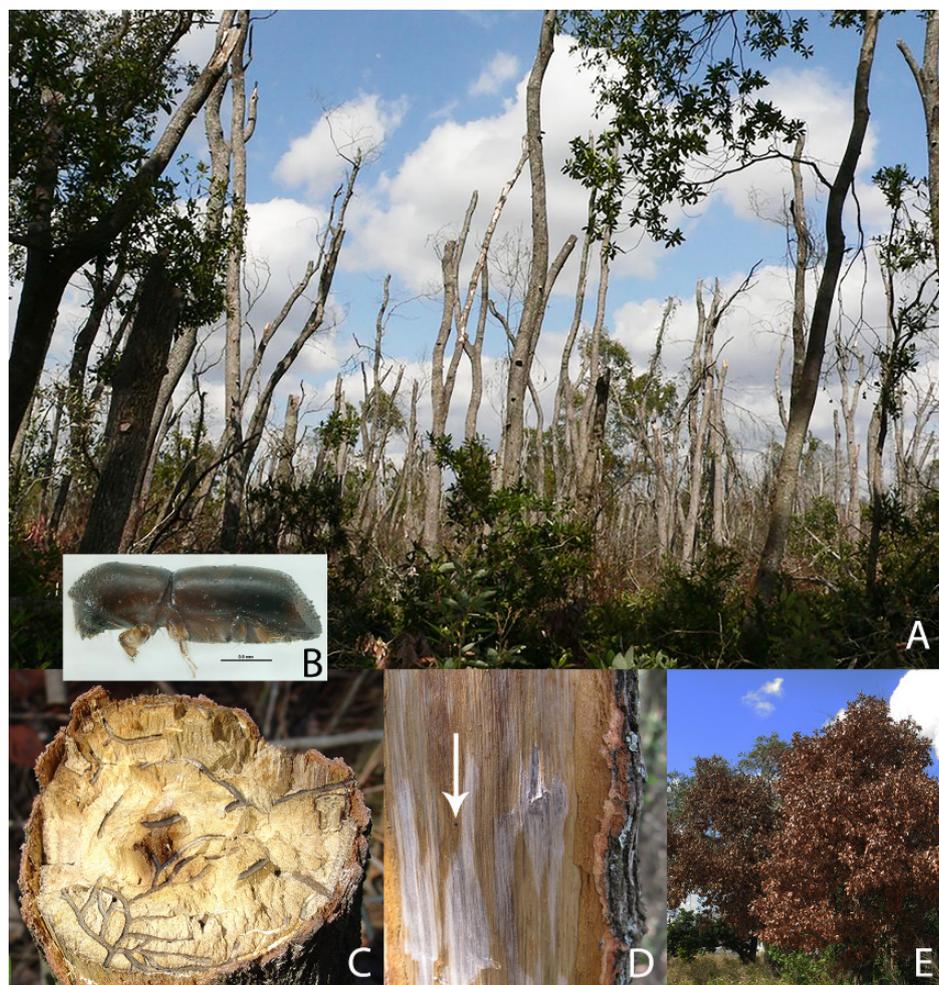


Figure 1. (A) Devastation caused by laurel wilt in a former stand of redbay, *Persea borbonia*, in Georgia in 2009 (photo: R. Ploetz). (B) Adult female of *Xyleborus glabratus* (PaDIL photo 33462, Justin Bartlett, <http://www.padil.gov.au/>). (C) Galleries of *X. glabratus* in a redbay tree that was killed by laurel wilt (photo: P. Kendra). (D) Internal staining of the sapwood of a redbay tree that is affected by laurel wilt (photo: R. Ploetz). Note inconspicuous entrance to a gallery of *X. glabratus* denoted with an arrow. During the early development of this disease, evidence for *X. glabratus* is limited. (E) Laurel wilt-induced death of redbay trees in Florida (photo: R. Ploetz). Laurel wilt-affected trees of native *Persea* spp. in the southeastern United States defoliate, as in A), only several months after dying.

Laurel wilt affects trees and shrubs in the Lauraceae (Laurales, Magnoliid complex). It is caused by a fungal symbiont, *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva (Ophiostomatales), of an invasive Asian ambrosia beetle, *Xyleborus glabratus* Eichhoff (Curculionidae: Scolytinae) [13] (Figure 1B,C). Starting in its putative epicenter of Port Wentworth, Georgia, United States (i.e., where *X. glabratus* was first detected in 2002) [14,15], the disease had spread by August 2016 as far west as 95° W, as far east as 78.5° W, as far north as 35° N and as far south as 25.5° N [16].

An estimated 300 million redbay trees have been killed by laurel wilt [17], and damage to the ecosystems that are associated with this important species has been documented or predicted [18–23]. Other common species in the southeastern United States, such as sassafras (*Sassafras albidum* (Nutt.) Nees), silk bay (*P. humilis* Nash), and swamp bay (*P. palustris* (Raf.) Sarg.), are decimated by the disease, as are threatened (pondspice, *Litsea aestivalis* (L.) Fernald) and endangered endemics (pondberry, *Lindera melissifolia* (Walter) Blume) [24–26]. Commercial production of a crop from MesoAmerica, avocado (*P. americana* Miller), was affected in Florida beginning in 2012, and that outbreak poses an increasingly serious threat to commercial production there and in other, currently unaffected areas [27,28]. Other lauraceous species from Europe and the United States are also susceptible, including bay laurel (*Laurus nobilis* L.) and, after artificial inoculation, California laurel (*Umbellularia californica* (Hook. and Arn.) Nutt.), gulf licaria (*Licaria triandra* (Sw.) Kosterm.), lancewood (*Nectandra coriacea* (Sw.) Griseb.), Northern spicebush (*Lindera benzoin* (L.) Blume) and Viñátigo (*P. indica* (L.) Spreng.) [13,26,29–32]. An Asian endemic, camphortree (*Cinnamomum camphora* (L.) J. Presl.), generally tolerates the disease [33].

2. Origins

Rabaglia et al. [15] indicated that *X. glabratus* was native to Asia and had been recorded from Bangladesh, India, Japan, Myanmar and Taiwan. Recently, Hulcr and Lou [34] reported the insect in mainland China. They confirmed that *X. glabratus* preferred lauraceous hosts (*Phoebe zhennan* S. Lee and F.N. Wei, *Machilus nanmu* Nees, *C. camphora* and *Phoebe neurantha* (Hemsl.) Gamble in China), and that taxa in other families were rarely colonized (only two of 40 collections).

Harrington et al. [35] recovered *R. lauricola* from specimens of *X. glabratus* from Japan and Taiwan. Since there are no reports of laurel wilt in the United States prior to 2004, *X. glabratus* probably carried the pathogen when it was first detected in Port Wentworth [14,15]. The focal and temporal spread of laurel wilt from that area (see [16]) and the genetically uniform populations of *R. lauricola* and *X. glabratus* that are found throughout the southeastern United States suggest that a single founding event, in or before 2002, may be responsible for the laurel wilt epidemic [17,36,37].

Non-Asian susceptibles in the Lauraceae are all naïve (new-encounter) hosts [1,26]. Despite the wide geographic range of *X. glabratus* and the large number of species in the Lauraceae that are endemic to Asia (see below), there is only one report of laurel wilt in Asia, and that was on the introduced non-Asian host, avocado [38]. Fraedrich et al. [33] indicated that “there are no reports that indicate *R. lauricola* causes a plant disease in Asia”, and Hulcr and Lou [34] doubted that (sic) “*X. glabratus* displays tree-killing behavior in its native range.” If laurel wilt occurs in Asia on Asian members of the Lauraceae, it must be inconspicuous.

3. Coevolution

The term “coevolution” was coined to describe butterfly x plant interactions [39]. However, the idea that reciprocal evolution occurred between sympatric species was discussed by Darwin [40] and described in a plant-pathological context in the 1950s. In describing results from his classic research on flax rust, Flor [41] suggested that “. . . obligate parasites, such as the rust fungi, must have evolved in association with their hosts” and that “. . . during their parallel evolution, host and parasite developed complementary genic systems”. Gene-for-gene and genetically quantitative/multi-gene systems have now been identified in many other pathosystems, and the specific adaptation of pathogens to host taxa, such as those described as *formae speciales*, is generally accepted as “the outcome of coevolution” [42]. These relationships can be conceived of as arms races in which increased disease resistance develops in a host in response to increased virulence in a pathogen [43,44].

Although these interactions can be difficult to document [43,45,46], coevolution appears to be an important factor in the development of many pathosystems [47]. Several criteria can be used to identify possible coevolved pathosystems [41–43,45,46,48–52]; they include:

1. A limited, often specific host range for the pathogen;
2. An original geographic distribution of the pathogen that overlaps with that of the host;
3. The occurrence of significant disease resistance in the host's primary center of origin;
4. Regional overlap of resistance and pathogenicity factors and phenotypes in the respective host and pathogen populations (i.e., geographic evidence for reciprocal selection);
5. Gene-for-gene relationships (although quantitative, non-gene-for-gene interactions can also co-evolve); and
6. Tandem speciation (also known as parallel cladogenesis).

Due to the rigorous criteria that are needed to confirm these relationships, it is not surprising that relatively few unequivocal examples of host x pathogen coevolution exist [51,52]. Most proposed coevolved pathosystems possess some, but not all, of the above attributes [10,42,53,54]. For example, although the first three of the above criteria are met for *R. lauricola* in Asia, there are no data for criteria 4–6. Future work may provide additional support for the idea that *R. lauricola* coevolved with endemic laurels in Asia. In the meantime, studies of Asian species in the Lauraceae, such as camphortree, could provide valuable insight into how tolerant hosts respond to this pathogen, and which attributes should be sought during the development of laurel wilt-resistant genotypes.

4. Ambrosial Symbioses

Xyleborine ambrosia beetles (Curculionidae:Scolytinae:Xyleborini) exhibit a haplodiploid, sibling mating system, which is also known as arrhenotoky [55–57]. Females are diploid and establish colonies after dispersion to uncolonized portions of the same or new host trees. Fertilized females can establish new colonies of females and haploid males, whereas nonfertilized females lay haploid eggs that become males. Males are flightless and rarely leave their natal galleries [58], where they mate with their mother and sisters.

Ambrosia beetles carry their fungal symbionts in specialized structures called mycangia [59–61]. In the Xyleborini, paired pre-oral (also known as mandibular) mycangia are small invaginations at mandible bases, mesothoracic mycangia are single, large invaginations between the meso- and metanotum, and elytral mycangia are small cavities at elytra bases [61,62]. In the species that have been examined, only one type of mycangium is present. However, mycangia are absent in some species that plunder fungal gardens of other species [63].

When adult females disperse to new trees, they bore brood galleries into host tree xylem, in which they cultivate gardens of the fungal symbionts. The developing colony feeds on these fungi (not wood), and as the colony matures new females are eventually produced. They then perpetuate the species by dispersing and establishing new colonies. Although some ambrosia beetle species (e.g., *Xylosandrus compactus* Eichhoff) can attack and colonize (establish brood colonies in) healthy trees [64,65], most reproduce only in stressed or dead trees.

During the early stages of the laurel wilt epidemic, Fraedrich et al. [13,33] examined *X. glabratus* x redbay interactions. They indicated that initial attacks by the insect in healthy trees were aborted and that reproduction by the insect was not observed in such trees. Nonetheless, aborted attacks were sufficient to infect trees with *R. lauricola*. Only after laurel wilt began to develop in infected trees was brood development by *X. glabratus* observed [13,33]. If this sequence is typical, *X. glabratus* may resemble other ambrosia beetle species in that it preferentially colonizes and reproduces in compromised or dead trees.

Most ambrosia beetles are generalists with wide host ranges. Thus, *X. glabratus* is unusual as it displays a strong preference for trees in the Lauraceae. Sesquiterpenes, rather than ethanol, the stress metabolite to which ambrosia beetles are usually attracted, appear to be a significant component of the attraction signature of these trees [66]. This difference has been cited when indicating that the *X. glabratus* vector relationship is exceptional, but there appears to be no evidence that trees identified with these signatures then support colonization and brood development before disease develops. Thus,

indications that the interaction of *X. glabratus* with its host trees is atypical [61,67,68] should be clarified. Clearly, better information on, and a distinction between, the early (attack phase) and mid-stage interactions of *X. glabratus* with its host trees (colonization and brood development) are needed.

In discussing the appearance of laurel wilt in the United States and its absence in the Asian homeland of *X. glabratus*, Hulcr and Dunn [68] proposed that “the sudden emergence of pathogenicity” was due to “a new evolutionary phenomenon.” A more parsimonious explanation for the emergence of laurel wilt is that coevolution with *R. lauricola* eliminated susceptible species in Asia, but not in the United States. The idea that American strains of *R. lauricola* became pathogenic after their move from Asia is not supported by data from studies that detected no differences in pathogenicity to avocado and swamp bay between isolates of *R. lauricola* from Asia and the United States [36,38]. Hulcr and Dunn [68] also suggested that an “olfactory mismatch” may be responsible for the identification by *X. glabratus* of nondiseased host trees as suitable for colonization. Since *X. glabratus* propagates in compromised host trees, as do other ambrosia beetle species, the attractive sesquiterpenes may simply enable *X. glabratus* to identify trees in which its ambrosial symbiont, *R. lauricola*, will establish, regardless of the host tree’s health status. In summary, pathological differences have not been evident between *R. lauricola* in Asia and the United States, and *X. glabratus* and other ambrosia beetles appear to have similar reproductive preferences for compromised or dead trees. However, no other ambrosial symbiont is known to be a systemic, lethal pathogen. Even when ambrosial symbionts kill trees (e.g., *R. quercus-mongolicae* K.H. Kim, Y.J. Choi and H.D. Shin, *R. quercivora* Kubono and Shin. Ito, and *Fusarium euwallaceae* S. Freeman, Z. Mendel, T. Aoki et O’Donnell), mortality is due to localized (nonsystemic) necrosis and multiple attacks by the associated ambrosia beetle vectors [1].

More work is needed to understand the impact of *R. lauricola* on naïve American hosts. Host tree colonization by *X. glabratus* is incompletely understood, and discerning the role that other ambrosia beetle species may play in the laurel wilt epidemic has only begun. Several other species are associated with redbay, avocado and other lauraceous hosts, but they have been considered as vectors of *R. lauricola* only recently. The general absence of *X. glabratus* in avocado orchards that are affected by laurel wilt, the pathogen’s presence in other species of ambrosia beetle that are recovered from avocado and other host trees, and the experimental demonstration of pathogen transmission and subsequent laurel wilt development in redbay and avocado suggest that species other than *X. glabratus* could play roles in the epidemiology of this disease [1,69,70].

5. Vectors of *Raffaelea lauricola*

Although ambrosia beetles have an obligate association with nutritional fungi [59,61,71,72], these can be promiscuous relationships wherein a given beetle species carries more than a single symbiont, and the same fungus species is present in more than one species of beetle. The movement of symbionts amongst ambrosia beetle species had been recognized previously [69,71,73,74]. However, the magnitude and speed with which this has occurred for *R. lauricola* is unprecedented [70]. Since its introduction into the United States in or before 2002, *R. lauricola* has been horizontally transferred from *X. glabratus* to nine additional ambrosia beetle species [1,69,70,75,76].

Reared (from laurel wilt-affected host trees) or trapped individuals (in the proximity of laurel wilt-affected trees) of 14 species of ambrosia beetle (*Ambrosiodmus*, *Euwallacea*, *Premnobius*, *Xyleborus*, *Xyleborinus* and *Xylosandrus* spp.) were assayed for *R. lauricola* by Ploetz et al. [70]. During 10 experiments, the pathogen was recovered from 34% (246 of 726) of the individuals that were associated with *Persea* spp. that are native to the southeastern United States, but only 6% (58 of 931) of those that were associated with avocado. *Raffaelea lauricola* was recovered from 10 of the ambrosia beetle species that were assayed, including *X. glabratus*, but was most prevalent in *Xyleborus* congeners [70]. Previous reports had suggested that *Raffaelea* spp. were the primary symbionts of *Xyleborus* spp. [74,75]. In general, mycangia of *X. glabratus* contained 10–1000 times more colony forming units (CFUs) of *R. lauricola* than the other assayed species. From native *Persea* spp. and avocado, *R. lauricola* was recovered from a respective 91% and 60% of the live specimens of *X. glabratus* that were assayed [70].

Although little is known about symbioses that are established between *R. lauricola* and different ambrosia beetle species, some of these insects may be involved in the ongoing epidemic in the southeastern United States [28,70]. In no-choice experiments, Carrillo et al. [69] reported that six and two species other than *X. glabratus* transmitted *R. lauricola* to potted redbay and avocado trees, and that laurel wilt developed in six and one of these interactions, respectively.

Kostovcik et al. [74] indicated that different types of mycangia may “support functionally and taxonomically distinct” symbioses. In summarizing a study of microbial communities in mycangia of *Xyleborus affinis* Eichhoff, *Xyleborus ferrugineus* Fabricius and *Xylosandrus crassiusculus* Motschulsky, they concluded that the mandibular (pre-oral) mycangium found in *Xyleborus* enabled the establishment of a broader array of symbionts than the larger and more exposed mesonotal mycangium possessed by *X. crassiusculus* (and *Xyleborinus saxesenii* Ratzeburg).

Hulcr et al. [77] studied how different ambrosia beetle species responded to *R. lauricola*. In olfactometer assays, *X. glabratus* was significantly attracted to *R. lauricola* in 54 of 84 assays ($p = 0.004$). In contrast, adult females of *X. saxesenii* and *X. crassiusculus* were significantly repelled by the fungus [77], which corresponds with its uncommon recovery from these species (1%–4% of all assayed individuals in [70]). Interestingly, another beetle that carried *R. lauricola* more frequently than *X. saxesenii* and *X. crassiusculus*, *X. ferrugineus* (11%–57% of the individuals in [70]), had a net nonresponse to the fungus; i.e., was repelled about as often (156 assays) as it was attracted (132 assays) ($p = 0.16$) [77].

Although beetle attraction to, or avoidance of, *R. lauricola* may impact whether it is a factor in the epidemiology of this disease [28,70], it is unclear whether species that are repelled by *R. lauricola* in olfactometers could still be occasional vectors of the pathogen and whether attracted or neutral species would disseminate the pathogen more frequently. Clearly, the involvement of other ambrosia beetle species in the laurel wilt epidemic requires more study. Nonetheless, we are beginning to understand the vector portion of this puzzle. By virtue of their affinity for *Raffaelea* spp. [70,74,75], species of *Xyleborus* might be expected to foster symbioses with *R. lauricola* more readily than species in other genera. In contrast, species with mesonotal mycangia, such as *X. crassiusculus* and *X. saxesenii*, should be able to carry greater quantities of the pathogen, based on the larger size of this organ compared to pre-oral mycangia. Even though they are infested with *R. lauricola* infrequently and are not attracted to the fungus in olfactometers, highly infested individuals with mesonotal mycangia could be vectors of *R. lauricola*.

6. Vector Chemical Ecology and Host Location

Host-based attractants (kairomones) have been studied most extensively for the primary vector of *R. lauricola*, dispersing females of *X. glabratus*. This beetle is not attracted to ethanol [78], the standard lure for ambrosia beetle detection [79]. The strongest female attractants identified to date are terpenoid kairomones, specifically volatile sesquiterpenes emitted from host wood [80–82]. In comparative studies with nine lauraceous species, emissions of four sesquiterpenes (α -copaene, α -cubebene, α -humulene, and calamenene) were positively correlated with in-flight attraction of *X. glabratus*, and electroantennography has confirmed olfactory chemoreception of these compounds [66]. A succession of field lures has been developed using essential oils naturally high in sesquiterpenes, including manuka oil (derived from *Leptospermum scoparium* J.R. Forst. and G. Forst., Myrtaceae; [80]), phoebe oil (*Phoebe porosa* (Nees and Martius) Barroso, Lauraceae; [80]), and cubeb oil (*Piper cubeba* L. f., Piperaceae; [83–86]). Laboratory bioassays with fractionated cubeb oil identified the negative enantiomer of α -copaene as a primary attractant, sufficient to evoke positive chemotaxis. Currently, the most effective lure for *X. glabratus* is an essential oil product enriched to 50% (–)- α -copaene content [87,88].

Exiting the natal tree to locate and colonize new resources is critical for the reproductive success of ambrosia beetles, but this brief dispersal event potentially exposes them to predation and adverse environmental conditions. To minimize risks associated with dispersion, it would be adaptive for

females to have efficient host-seeking behaviors, guided by reliable cues. Kendra et al. [66] proposed that host location and acceptance is a multistep process directed by a series of cues presented in sequential order.

Initiation of dispersal flight in scolytine beetles is determined by light intensity, temperature, relative humidity and other environmental cues ([89] and references therein). With *X. glabratus*, females engage in host-seeking flight during the late afternoon and early evening, several hours earlier than other species of *Xyleborus* in Florida [90]. While in flight, females orient initially toward long-range olfactory cues; α -copaene appears to be the primary kairomone, but other terpenoids likely contribute to generate an attractive “signature bouquet” of the Lauraceae [66,82,87]. In addition to sesquiterpenes, several monoterpenes have been reported as kairomones, including eucalyptol (1,8 cineole) [91] and a blend of redbay leaf volatiles [92]. There is no evidence that *X. glabratus* utilizes sex or aggregation pheromones [78].

As females approach the focal source of host kairomones, visual cues assist in directing flight toward individual trees. Mayfield and Brownie [93] demonstrated that stem silhouette diameter aids in host location, but only when presented in an appropriate chemical context (i.e., presented concurrently with host odors). These experimental data support field observations that the oldest, largest-diameter trees are typically the first to be attacked by *X. glabratus*. In addition, there is a higher density of beetle entrance holes on the trunk and large diameter branches of redbay and swampbay, compared to smaller diameter branches [10,58,84]. In mature avocado trees, the highest emissions of α -copaene and α -cubebene are detected on the trunk and larger branches [94]. This chemical gradient may further assist with location of optimal sites for landing and initiation of a gallery. Even though large hosts are preferred, once those resources are depleted, smaller diameter trees are utilized, thereby enabling low populations of *X. glabratus* to persist for many years [95].

The short-range cues that prompt a shift from host-seeking to host acceptance and boring behaviors have received scant study. Knowledge in this area could facilitate the development of effective repellents or boring deterrents. Once a female makes contact with a potential host, she likely integrates a variety of stimuli, including olfactory, gustatory, contact chemosensory, tactile and visual cues, all of which must reinforce the message that an appropriate host has been found before a reproductive effort is initiated. In short-range laboratory bioassays, *X. glabratus* is attracted to volatiles emitted from its fungal symbiont [77], and in field tests these volatiles increased beetle captures when combined with host-based lures [96]. The ability to detect food-based odors may be adaptive in the host-choice process, confirming that the tree under evaluation is capable of supporting growth of required (nutritional) fungal resources.

7. Pathogen Attributes

The fungal symbionts of ambrosia beetles typically colonize only the lining of the natal gallery. In rare cases, these fungi cause serious damage in host trees, for example, *R. quercus-mongolicae* and *R. quercivora* on *Quercus* spp., and *F. euwallaceae* on diverse host species [1,97,98]. However, in these and other cases in which significant damage occurs, the fungal symbiont does not move systemically in host xylem and causes only localized damage; in these situations, tree mortality is associated with mass attack by the beetle vectors. *Raffaelea lauricola* is a unique symbiont, in that it systemically infects tree xylem and a single infection of susceptible trees can be fatal.

The population of *R. lauricola* in the United States is genetically uniform, apparently resulting from a single founding event (perhaps the importation of *X. glabratus* to Port Wentworth, Georgia). Little to no genetic variation was detected in isolates of the pathogen across the southeastern United States when Amplified Fragment Length Polymorphisms (AFLPs) and microsatellite markers were used [17]. With the same microsatellite markers and sequences of the large subunit of ribosomal (LSU) DNA, Wuest et al. [37] also concluded that the population of *R. lauricola* in the United States was quite uniform, in contrast to far greater diversity that they detected in the pathogen in Japan and Taiwan. When isolates from Asia were tested on avocado and swamp bay, they were as pathogenic as isolates

from the United States [36,38]. Thus, although greater genetic diversity in *R. lauricola* has been found in Asia, differences in pathogenicity have not been apparent when isolates from the two regions have been compared.

The role of symbiont pathogenicity in the ecology of ambrosia beetles is not well understood [1,99]. Evident by comparison with other introductions is that the aggressiveness by which the laurel wilt pathogen systemically colonizes its North American tree hosts and causes lethal vascular wilt is an extreme scenario [1]. The range of fungal-specific damage observed among ambrosia symbionts in their native habitats varies from asymptomatic (nonpathogenic) to localized damage and host recovery (mildly virulent).

From the perspective of fungal pathogenicity, two hypotheses may help to understand the extreme aggressiveness of *R. lauricola* on North American Lauraceae. The first of these is that *R. lauricola* is an “accidental pathogen”. Adaptations that enable *R. lauricola* to colonize natal galleries may, when encountered beyond the coevolved range of hosts, result in a massive host defense response that ultimately leads to xylem dysfunction and host death. The second hypothesis is that pathogenicity is a selective force in Asia that is kept in balance by coevolutionary processes; susceptible hosts, such as the naïve laurels in the southeastern United States, are eliminated, whereas those that tolerate infection persist and eventually replace their susceptible relatives.

Tests of these hypotheses are underway by comparing genomes of *R. lauricola* and its close relatives [100]. These analyses are aided by recently published phylogenies of *Raffaelea* [101,102], which have identified the closest, extant relatives of *R. lauricola*; notably, all other members of the phylogenetic clade in which *R. lauricola* resides (*Raffaelea sensu stricto*) are not plant pathogens. Of particular interest are genome-wide comparisons between *R. lauricola* and its close relative *R. aguacate* D. R. Simmons, Dreaden and Ploetz. *Raffaelea aguacate* has only been recovered from avocado [102], but it is neither systemic nor pathogenic on avocado or redbay [103]. Evidence for the accidental versus adapted pathogen hypotheses is being sought in comparative analyses between these two genomes and among genomes of related species. Virulence gene content, including those for putative effectors, elicitors and toxins, will be assessed, as will the expansion of these pathogenicity-associated gene families, and evidence for diversifying selection among candidate virulence factors.

Unraveling these fundamental questions may provide significant insight and predictive value for what might be expected in the future. If all ambrosial symbionts have the potential to be plant pathogens, is it a matter of time before another beetle–fungus–tree combination facilitates the emergence of another laurel wilt-like pathogen (“symbiont roulette”)? If, on the other hand, pathogenicity is a derived character, what are the attributes required for weakly pathogenic symbionts to be aggressive pathogens when introduced outside their native range?

8. Hosts of Laurel Wilt

The Lauraceae is a large family that includes over 50 genera and 2500 to 3000 species [104]. The family represents some of the earliest angiosperms and has a fossil record dating back to the Mid-Cretaceous [105]. It is well represented on both sides of the Pacific Basin, and the so-called “amphi-Pacific tropical disjunction” of the *Persea* and *Cinnamomum* groups in the family has been examined to understand the presence, and the origins and relatedness, of family members in the Eastern and Western Hemispheres [101,106,107].

Although there are notable exceptions, such as naturalized populations of camphortree in the southeastern USA and the globally cultivated crop, avocado [108,109], most species in the family have restricted distributions. In the American tropics, lauraceous taxa comprise significant portions of lowland forests and montane environments [110,111]. Considering their ecological importance, laurel wilt could have an even greater impact as it spreads to new areas in the Western Hemisphere.

Of economic concern is the potential impact of the disease in currently unaffected avocado-production areas [28]. Avocado is a subtropical/tropical tree, and a significant fruit crop. In 2014, 5 million metric tonnes (MMT) of fruit were harvested worldwide, and Mexico was the

leading producer (1.5 MMT) and exporter (in 2013, 0.6 MMT valued at ca. US\$1.1 billion) [112]. Depending on the cultivars that are grown, which vary considerably in cold tolerance, the crop is grown commercially from United States Department of Agriculture Hardiness Zones 10 to 11, with moderate urban production of some cultivars occurring into Zone 9. Florida's avocado industry is the state's second-largest fruit industry after citrus, and 85% of the producers have orchards of less than 13 hectares. In the United States, more than 6600 growers on mostly small farms (less than 45 hectares) produce avocado; annual production worth more \$1.6 billion is at risk [112].

Laurel wilt has affected avocado in Florida for at least a decade, but so far only ca. 3% of the avocado trees in commercial production have been killed [113]. However, the short time laurel wilt has been in South Florida (where most commercial production occurs), the great susceptibility of avocado to this disease, and experience with other lethal, invasive diseases [11,114] suggests that laurel wilt will, in the future, cause increasing damage on avocado in Florida. Laurel wilt imperils avocado production in other states (e.g., California and Hawaii), USA protectorates (Puerto Rico) and neighboring countries such as Mexico [28].

Persea americana is divided into Mexican (M), Guatemalan (G) and West Indian (WI) (also known as Lowland or Antillean) botanical races, respectively vars. *drymifolia* (Schltdl. and Cham.) S.F. Blake, *guatemalensis* (L. O. Williams) Scora, and *americana* Mill. [115]. Hybridization occurs freely among the races and is associated with a range of responses to calcareous soil, salinity, high and low temperatures, and other stresses [109,116]. Different responses to laurel wilt have also been noted among the races and racial combinations of the species; in general, greater susceptibility occurs in WI cultivars [117,118].

Although the most serious outbreaks of laurel wilt have occurred on lauraceous natives from the southeastern United States, a few natives from the region, gulf licaria and lancewood, display moderate tolerance after artificial inoculation [32,119]. They develop vascular symptoms, but do not die, which is similar to the response of camphortree [33]. Notably, rare tolerance to laurel wilt has been selected in redbay, and there is hope that these selections could be used to re-establish this tree in the southeastern coastal plain [36,120].

More data are needed on the susceptibility of American and non-American species in the family, and how susceptible and resistant hosts respond to infection by *R. lauricola*. To date, we have only rudimentary understandings of these processes [33,121–123]. Although natural selection against susceptibility has probably occurred in camphortree and other Asian Lauraceae [1,33], host attributes that are associated with laurel wilt tolerance have been studied only recently [121].

9. Host Responses to Infection by *Raffaelea lauricola*

Vascular wilt diseases typically exhibit wilting, sapwood discoloration (Figure 1D) and vascular dysfunction associated with physical and histological changes in the host [124]. The production of gels [125] and tyloses [126] in the xylem are two of the most common attributes of affected woody plants [127–131].

Tyloses are formed in xylem lumina in response to pathogen infection, embolism, aging, and injury [131]. They are outgrowths from adjacent parenchyma cells, and even though they can prevent desiccation, damage, and infection of adjacent cells [131], they also reduce hydraulic conductivity (water conductance) [132]. Gels induced by pathogens generally arise from host perforation plates, end walls, and pit membranes of the primary wall and middle lamella [131,133,134]. Breakdown of these cellular components by the pathogen results in the accumulation of gels [135].

Symptoms of laurel wilt on avocado, redbay, and swampbay include rapid wilting of foliage and vascular discoloration (Figures 1D,E and 2). Xylem blockage associated with tylose and gel formation appears to be at least partially responsible for the wilting symptoms [122,123]. Xylem function (the ability to conduct water) in avocado is impaired as soon as 3 days after inoculation, before the development of external or internal symptoms of the disease are apparent [123] (Figure 3). Tree mortality is associated with vascular functionalities of less than 10%.

Artificial inoculations with as few as 100 conidia of *R. lauricola* can kill avocado and swamp bay [136]. After laurel wilt-susceptible (avocado and swamp bay) and tolerant (camphortree) hosts were inoculated with a green fluorescent protein (GFP)-labelled strain of *R. lauricola*, Campbell et al. [120] reported that the pathogen was scarcely visible in microscopic cross sections, even in dead or dying plants. Although they observed that a maximum of 0.9% of the xylem lumina of avocado were colonized by the GFP-labelled strain 30 days after inoculation (dai) (Figure 4), about 40% of the lumina of avocado were occluded by tyloses 21 dai in another study [123] (Figure 5). Mobility of the pathogen or its metabolites in the xylem seems to be related to susceptibility.

Symptom development has been associated with reduced water transport in other, similar tree diseases. Wych elm affected by Dutch elm disease (caused by *Ophiostoma novo-ulmi* Brasier) [137], bitternut hickory affected by hickory decline (*Ceratocystis smalleyi* J. A. Johnson and T. C. Harr. [138], *Quercus* spp. affected by Japanese oak wilt (*R. quercivora*) [139], and *Notholithocarpus* (formerly *Lithocarpus*) *densiflorus* (Hook. and Arn.) Manos, Cannon and S.H.Oh affected by sudden oak death (*Phytophthora ramorum* Werres et al.) [140] all exhibited reduced xylem function which was, in turn, correlated with symptom development. For example, Park et al. [138] detected an inverse relationship between xylem sap flow and sapwood infection by *C. smalleyi*. They proposed that tylose formation induced by infection was responsible for reduced water transport in affected trees; *C. smalleyi* caused multiple cankers and reduced xylem function, resulting in crown wilt and decline of bitternut hickory.

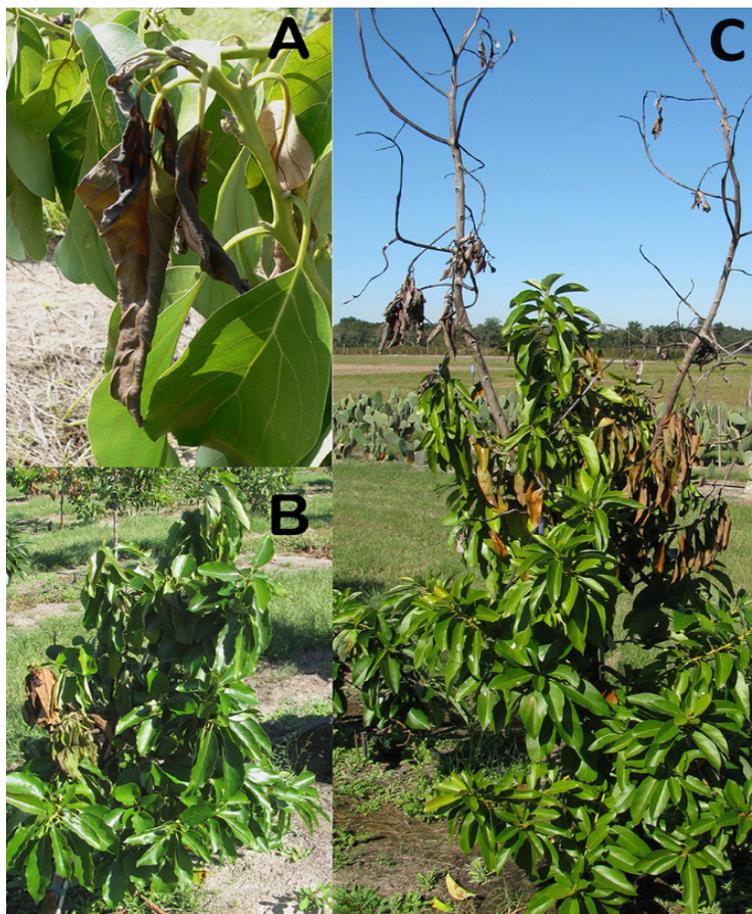


Figure 2. Foliar symptoms of laurel wilt on avocado include (A) wilting and an oily grey discoloration that rapidly progresses to necrosis; (B) initial symptom development in only a portion of a tree's canopy, involving a limited number of vascular traces; and (C) involvement of greater portions of the canopy followed by defoliation within a month or two of symptom onset (far more rapidly than occurs in redbay and other native *Persea* spp.) (photos: R. Ploetz).

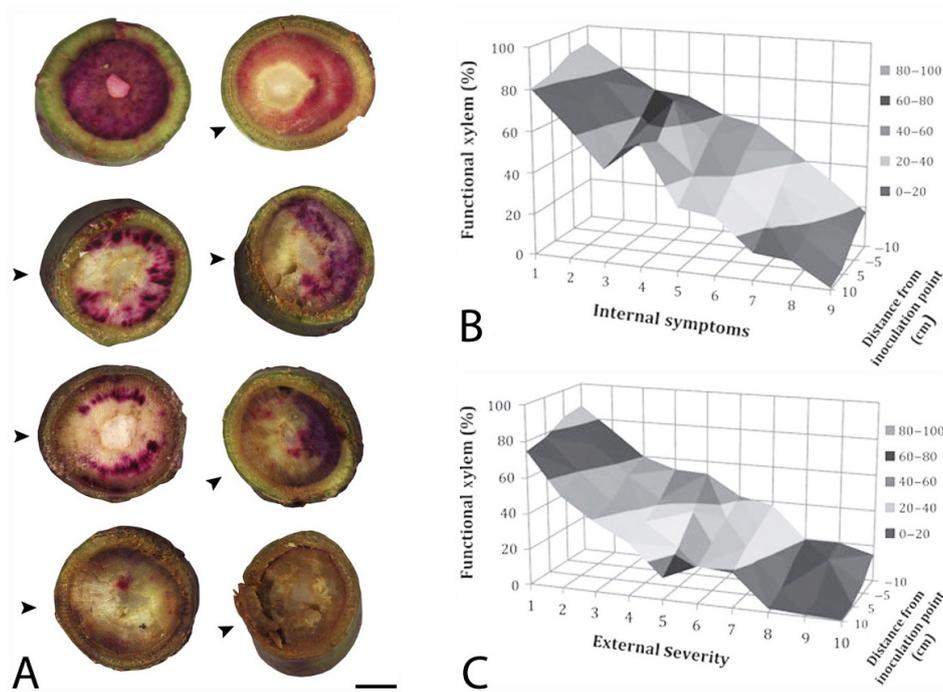


Figure 3. “Simmonds” avocado trees were inoculated with *Raffaelea lauricola* and xylem function was assessed with an acid fuchsin stain [122]. In (A), burgundy to pink staining in stem sections indicates functional xylem, which was quantified in scanned, digital images. From left to right and top to bottom, xylem in cross sections are: 98% functional (water control, internal symptoms (is) = 1; 86% (3 days after inoculation (dai)), is = 1; 76% (7 dai, is = 2); 71% (14 dai, is = 3); 32% (21 dai, is = 5); 30% (21 dai, is = 6; 5% (42 dai, is = 9; and 1% (42 dai, is = 9). Arrows (◀) indicate the position on a stem that was inoculated, and the scale bar = 0.5 cm. In the response surfaces, % of functional xylem is graphed on the y-axes against the distance from the inoculation point on the z-axes and (B) internal and (C) external symptom development on the x-axes, which was rated on a 1–10 scale, where 1 = healthy, no symptoms, and 10 = dead, totally symptomatic.

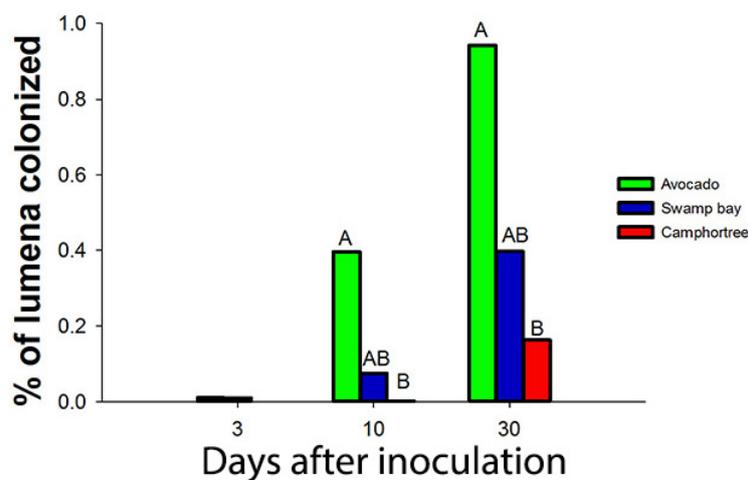


Figure 4. Campbell et al. [121] observed that less than 1% of the lumina of avocado, swamp bay and camphortree were colonized with a GFP-labelled strain of *Raffaelea lauricola*, even 30 days after inoculation (dai) (when most plants of avocado and swamp bay had died from laurel wilt).

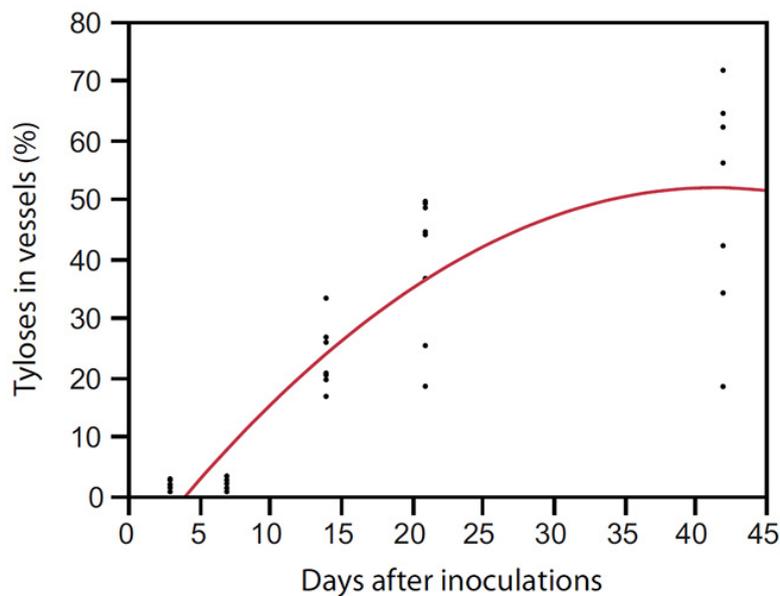


Figure 5. “Simmonds” avocado was artificially inoculated with *Raffaelea lauricola* and xylem lumina were examined in stem cross sections for tylose formation 7, 14 and 42 days after inoculation ($R^2 = 0.78$, $y = -0.69 + 1.79x - 0.04(x - 17.4)^2$, $p < 0.0001$) [123].

Ploetz et al. [118] hypothesized that an avocado scion’s susceptibility to laurel wilt is related to its ability to conduct water. They observed that pre-inoculation sap flow rates were greater ($p = 0.05$) in a susceptible cultivar, “Russell” WI, compared to the less susceptible cultivars “Brogdon” M \times G \times WI and “Marcus Pumpkin” G; however, sap flow plummeted in “Russell” soon after inoculation. Recently, Campbell et al. [121] reported that cross sections of xylem lumina of “Simmonds”, another WI avocado cultivar, were nearly 2.5 times larger ($3063 \mu\text{m}^2 \pm 226$) than those of camphortree ($1250 \mu\text{m}^2 \pm 221$). Although they indicated that wood chemistry might impede the development of *R. lauricola* in camphortree (it contains antifungal compounds [141]), they also observed that smaller lumina in camphortree could hinder the movement of *R. lauricola* compared to host trees with larger lumina.

In long-term studies to develop elms that resist Dutch elm disease, vessel size and sap flow were reported to be key factors associated with the susceptibility of *Ulmus minor* Mill. and *Ulmus* hybrids [142–144]. Susceptible trees had significantly wider and longer vessels than those that resisted the disease [144]. Solla and Gil [142] hypothesized that vessel diameters and sap flow affected the dispersal of *O. novo-ulmi* in the elm host, and Venturas et al. [144] suggested that xylem structure restricted the pathogen’s spread in resistant elms.

Additional work is needed to understand the relationship between susceptibility to laurel wilt and sap flow and other xylem-associated attributes, as it may facilitate the identification of laurel wilt-tolerant genotypes of host trees. Whether sap flow rates and xylem attributes are general predictors of laurel wilt susceptibility and tolerance in avocado are examined in ongoing work.

10. Ecology and Epidemiology

To date, native *Persea* spp. (redbay, swamp bay and silk bay, which some refer to collectively as redbay) have been most severely impacted by laurel wilt. Within a few years of affecting a stand, most mature trees of these species succumb to this disease [13,21,22,145,146]. Symptoms of laurel wilt develop soon after host trees are infected by *R. lauricola* (usually within 2 to 3 weeks of artificial inoculation) [117]. As the disease develops, host trees increase the production of volatiles that attract *X. glabratus* [92], which then promotes mass attack, colonization and brood development by *X. glabratus*.

The explosive nature of the laurel wilt epidemic is due to the great susceptibility of the native *Persea* spp. [26], their attractiveness to *X. glabratus* [66,78], the rapid increase in *X. glabratus* numbers

in affected stands of these trees [58], and the substantial amounts of inoculum that most females of *X. glabratus* carry [70]. Furthermore, tree-to-tree spread via interconnected root systems, which has been suspected in avocado [28] and sassafras [147], and demonstrated in pondberry [148], enables movement of the pathogen without vector assistance. It is hard to imagine a more efficient means for killing trees than this disease.

Raffaelea lauricola typically infects avocado only via ambrosia beetle vectors and interconnecting root grafts (see below), although other avenues of infection have been studied experimentally. Mechanical transmission of the pathogen was possible with artificially infested handsaws on potted plants, but the pathogen did not survive on circular saws that are used to prune avocado trees in commercial groves, presumably due to the high heat that was generated on these blades during use [149]. Seed and scion transmission of *R. lauricola* have also been discounted. After potted, fruit-bearing trees were artificially inoculated with *R. lauricola* plants were systemically colonized by the fungus, but in no instance did infection progress further than the hilum of fruit (87 fruit and their associated pedicels) [150]. Furthermore, when scions from artificially infected trees were used as grafting material, they did not establish on recipient rootstocks [151].

Most commercial avocado production in the eastern United States is located in Miami-Dade County in southern Florida; it is bordered on the north and east by metropolitan Miami and on the west and south by Everglades National Park (ENP) [152,153]. The proximity of a densely populated urban area and a protected natural area creates significant challenges for managing this disease.

Residential avocado trees are common in Miami. In other pathosystems, residential trees can act as reservoirs for pathogens from which neighboring agricultural areas are colonized. For example, residential trees played a significant role in epidemics of citrus canker in Florida and plum pox in Canada [154,155]. Residential trees are also associated with the regional spread of pathogens via the nursery trade, as infected asymptomatic plants can be sold and distributed through this network [156,157]. In this case, the short latent period of laurel wilt on susceptible hosts may be advantageous, as asymptomatic infection of these species would probably play a negligible role in spreading the disease. In contrast, tolerant species, such as camphortree, could serve as symptomless reservoirs of *R. lauricola* and *X. glabratus* in the landscape as well as a means by which the disease could spread; greater understanding of their potential role in the regional dissemination of this disease is needed.

Natural areas that border avocado production areas in southern Florida may present risks similar to those of residential avocado trees. Prior to the laurel wilt epidemic, swamp bay occurred throughout the ENP. Swamp bay is highly susceptible to laurel wilt [26] and is attractive to, and supports significant egg production of, *X. glabratus* [158]. An aerial survey of the Everglades in 2011 and 2013 detected rapid spread of laurel wilt on this host tree [25]. Since source-to-sink dynamics from unmanaged areas to agricultural areas have played an important role in other diseases with insect vectors [159], there was concern that swamp bay trees might act as disease and vector refugia in the ENP. However, recent spatial analyses suggested that laurel wilt outbreaks in the avocado production area are concentrated there, and that there is little connectivity between laurel wilt in the avocado and natural areas [160].

Once laurel wilt has established in an avocado orchard, it apparently moves among trees through root grafts [28]. Root grafting (i.e., the establishment of functional unions between the roots of different plants), is a common phenomenon in trees [161,162], and it plays a significant role in the movement of similar plant pathogens, such as those that cause oak wilt (*Ceratocystis fagacearum* (Bretz) Hunt) and Dutch elm disease (*Ophiostoma* spp.) [163–165]. In avocado orchards, high densities of trees are planted in rows in which root grafting occurs among adjacent trees. Previously, the root-graft movement of herbicides and another avocado pathogen, *Avocado sunblotch viroid* (ASBVd), were recognized [117,166–168]. Subsequent evidence that root-graft transmission of *R. lauricola* occurs has included the natural infection of roots by the pathogen, rapid expansion of disease foci, and the fact that prompt fungicide treatment of trees adjacent to diseased trees impedes spread and expansion of foci [28,117].

Preventing root-graft spread in avocado orchards is difficult. Since trenching to sever root grafts stops the spread of oak wilt and Dutch elm disease, it would presumably be useful in laurel wilt-affected avocado orchards. However, trenching would damage irrigation systems that are typically in place in avocado orchards. Other techniques to limit root graft spread are being explored, such as establishing barriers to pathogen movement with fungicide or herbicide treatments [28,169]. Since root grafts tend to develop in older plants, maintaining younger orchards and smaller trees might also inhibit transmission among trees.

While root-graft transmission enables rapid movement of the pathogen among trees, transmission also occurs among and within orchards via ambrosia beetles. As discussed above, *X. glabratus* is most important in natural settings, as it predominates in both trapping and rearing assays with these host trees [26,28,66], carries the greatest amounts of the pathogen [70], and was most consistently associated with transmission in no-choice assays [69]. However, the role that this species and its relatives play in the avocado system requires further study. The above spatial analyses [160] suggest that laurel wilt in swamp bay populations in the Everglades (on which *X. glabratus* would predominate) plays an insignificant role in the avocado epidemic. All of these data suggest that a complex of beetles, which may or may not include *X. glabratus*, are involved in laurel wilt dissemination in avocado. These beetles are understudied, and relatively little is known about their habits and roles in the environment. Understanding the ecological drivers of these insects is critical for future assessments of disease risk.

An early epidemiological model suggested that *X. glabratus* would not reach the southern tip of Florida until approximately 2015 [170]. However, *X. glabratus* was observed in Miami-Dade County in early 2010, and laurel wilt was reported in 2011 [153]. While this model accounted for host density and climatic features that benefit *X. glabratus*, it likely underestimated the effects of anthropogenic spread [170].

Although assigning an anthropogenic role to an outbreak of laurel wilt can be difficult, large geographic jumps in the disease's distribution and the close proximity of an outbreak to parks or other places where firewood is used, or where wood or wood products are handled, are attributes that suggest human agency. For example, Cameron et al. [171] reported outbreaks of laurel wilt in Georgia near a pulp mill and mulch plant. They also noted an apparent firewood-related outbreak near a campsite 120 km from the nearest outbreak. As this disease has spread, others have noted large jumps in its distribution. Riggins et al [172] reported a laurel wilt outbreak in Mississippi, over 500 km from the nearest known outbreak, and a recent finding in Texas was 300 km from the nearest known occurrence [16]. The above findings have been on redbay, but sassafras has also been impacted. Bates et al. [173] noted an outbreak on sassafras in Alabama, 160 km from the nearest outbreak, Fraedrich et al. [174] described an outbreak in Louisiana, 400 km from the nearest outbreak, and Olatinwo et al. [175] described a subsequent finding in Arkansas, 134 km north of the Louisiana outbreak. These range expansions suggest that anthropogenic spread is an important factor in the epidemiology of this disease. In addition, the increased prevalence of laurel wilt on sassafras, a common species that extends into Canada and which supports propagation of *X. glabratus*, indicates that the disease has the potential to continue its northward movement.

Effective landscape modeling of laurel wilt will need to account for biological (e.g., host range, host density and vector competency) and social factors (e.g., proximity to campgrounds and areas in which wood or wood products are handled). Integrating these factors is critical for understanding long-term disease prevention and control. Shearman et al. [176] used forest inventory and analysis data to estimate risk factors for redbay. They determined that the presence of laurel wilt in a county increased the odds that a given tree would be killed by approximately 154%, for every year after the initial outbreak, and that mortality rates increased by 5% for increases of 1 cm diameter breast height (DBH) in a tree's trunk diameter.

Epidemic network analysis is becoming a common part of the epidemiological toolbox [177,178]. Harwood et al. [179] used network analysis to evaluate likely outcomes for epidemics caused by

P. ramorum and *P. kernoviae* in the United Kingdom, as influenced by inspection strategies. These pathogens can spread through natural systems, as well as the nursery trade. They concluded that if the rates at which these pathogens were introduced increased, inspection activities could not keep up with the influx. Network analysis has also been used to identify key sampling locations for tracking epidemic progress [180,181]. For laurel wilt, spread via infested wood is a key vulnerability for long-distance movement, but this activity is difficult to monitor. To the extent that epidemic network structures can be characterized for a disease such as laurel wilt, strategies may be implemented to make inspection more efficient.

Impact Network Analysis [182] is a multilayer network analysis that uses agent-based modeling [183] to evaluate the likely system-level effects of regional management implementation. Multilayer networks integrate linked processes, such as networks of the spread of information about disease and linked networks for the spread of the disease and/or its vectors [180,184–186]. Multilayer networks can provide analyses about how networks interact to produce system level outcomes.

In Impact Network Analysis, three components are emphasized [182]. The first is a management concept or tool, such as a recommended cultural practice, a resistant variety, or effective pesticide, which is associated with a level of efficacy, a level of confidence in the estimated efficacy, and the cost of implementation. The second component is the network of communication and influence among managers who decide whether to implement the management tool [187], as well as the management landscape (e.g., natural or agro-ecosystem) in which management is or is not applied. The third component incorporates information on the epidemic network. Notably, establishment of a given disease in a new location may be strongly influenced by the management landscape. Impact Network Analysis is a platform for evaluating how a proposed management type or portfolio of management types is likely to influence regional epidemics, including the effects of manager decision-making [182].

An Impact Network Analysis is being developed for the laurel wilt epidemic in Florida and beyond. When the first component of the analysis is considered, the tools that are available for management are somewhat disheartening (see below). Currently, in natural systems that are populated by highly susceptible host species, there are no viable measures. Furthermore, although there are effective tools for avocado production, they are not consistently adopted by producers. The second component of the analysis, the network of communication and influence among managers, presents additional challenges. For example, some avocado managers may not be convinced of the importance of management, or may be motivated to manage only minimally until land is diverted for other purposes. The third component, the epidemic network, indicates that unmanaged outbreaks of laurel wilt are important risk factors in the avocado system. Growers who do not effectively manage laurel wilt may increase the difficulty with which the disease is managed by other avocado producers. Additionally, the impact of residential avocado production is a poorly understood, but potentially important facet of the epidemic network.

Serious challenges exist for managing laurel wilt in most situations. However, if laurel wilt epidemics in natural systems are shown to exert little or no influence on the avocado system, regional avocado management strategies could be simplified. Understanding the regional system will be a first step toward scaling up [188] risk assessment and management strategies in Cuba, Mexico and neighboring avocado-production areas.

Early detection of laurel wilt (ideally *R. lauricola*-infected trees before they develop symptoms) is critical to the containment of laurel wilt and the success of disease management efforts in avocado orchards. Since symptoms of laurel wilt can be confused with those associated with other biotic and abiotic factors, the presence of *R. lauricola* should be confirmed when a laurel wilt diagnosis is in doubt [26,28]. Currently, a taxon-specific method can be used to identify the pathogen and distinguish it from its closest relative, *R. aguacate* [102,189].

Visible–near infrared spectroscopy has been tested for the nondestructive detection of laurel wilt on avocado [190]. Classification studies were conducted with visible near infrared spectra of asymptomatic and symptomatic leaves from plants artificially infected with *R. lauricola*, as well

as leaves from noninfected freeze-damaged and healthy plants. Scores from principal component analyses were used as input features in four classifiers: linear discriminant analysis, quadratic discriminant analysis, Naïve-Bayes classifier, and bagged decision trees. All of the classifiers were able to discriminate leaves from plants with laurel wilt from freeze-damaged leaves. False negatives resulted mainly from asymptomatic leaves from infected plants being classified as healthy.

In subsequent work, spectral data were used to distinguish healthy, laurel wilt-affected, and *Phytophthora* root rot-affected avocado trees [191]. With a modified camera, spectral images were taken during helicopter surveys of commercial avocado orchards [192]. RmodGB digital data were used to calculate vegetation indices (VIs), band ratios, and VI combinations for healthy and laurel wilt-affected trees. Significant differences were observed in all VIs calculated among laurel wilt affected and healthy trees, although the best results were achieved with Excess Red, (Red–Green) and Combination 1. These results were used to modify a MCA-6 Tetracam camera with different spectral filters (580–10 nm, 650–10 nm, 740–10 nm, 750–10 nm, 760–10 nm and 850–40 nm), which was then used to take multispectral images of avocado trees at early, intermediate and late stages of laurel wilt development at three altitudes (180, 250 and 300 m) [191]. Inexpensive devices that use this technology need to be developed.

Canine detection was recently promoted for detecting avocado trees infected with *R. lauricola*, prior to the development of laurel wilt symptoms [193]. Although there has been some success in detecting other diseases under controlled settings [194], data for canine detection of laurel wilt has only been presented in seminars (no peer reviewed publications are available). Recently, canine detection was reported in avocado trees that displayed no obvious symptoms of laurel wilt [195]. Although *R. lauricola* was not isolated from 42% of the “detected” trees, in another avocado orchard, laurel wilt subsequently developed in most of the detected trees. Thus, trees from which the pathogen had not been isolated were, presumably, infected with *R. lauricola* when detected by dogs.

The reliability of canine detection requires additional evaluations. For example, the ability of dogs to distinguish infection in root-grafted trees, detect the pathogen prior to symptom development (see [196]), and distinguish *R. lauricola* from its close relatives, many of which are prevalent in ambrosia beetles that infest avocado [102,197], should be determined. Nonetheless, the available evidence suggests that canine detection could be a useful tool for managing laurel wilt in avocado orchards.

As alluded to above, the spread of laurel wilt is affected by poorly understood social factors. For example, even though early detection could help stem its spread the avocado system, producers might not adopt the available techniques (the second component in the above Impact Network Analysis). Furthermore, early detection would only be effective if detected trees were then promptly removed (sanitation) or treated with fungicide. Agricultural producers respond to uncertainty in different ways, and only a subset of all producers will adopt a given technology, no matter how effective it might be. Better understandings are needed for the impacts of different social, economic, and cognitive factors on the decision making process [183,198].

Laurel wilt management in commercial avocado orchards is possible, but difficult [199]. Effective fungicide treatments have been described, but they are expensive and their use may not be sustainable. Annual retreatment is indicated for even the best fungicide treatments. It becomes increasingly difficult to retreat trees as they heal from previous applications (wound tissue impedes fungicide uptake in previously treated sites). Subsequent applications are forced to move above the root collar, which is the most effective application site. Early detection and the rapid removal and destruction of affected trees is a more effective measure for managing this disease, but its adoption varies widely among producers. Retaining infected trees in an orchard is a dangerous, but common practice, as many producers are averse to removing trees that might produce fruit. Once focal development of the disease (root-graft movement of the pathogen) begins, laurel wilt becomes incredibly difficult to manage. In general, avocado producers who have been forced out of production by laurel wilt have practiced tardy and insufficient management.

In natural environments that are impacted by laurel wilt, the above management strategies are not useful; in these situations, eradication of the disease is not possible, sanitation has been ineffective when it has been used, and fungicide treatment is far too expensive [26,200]. Nonetheless, laurel wilt-tolerant individuals of redbay have been identified [120]. Although it would be a long-term proposition, these selections might be useful for re-establishing devastated populations of this important species.

11. Outlook

Laurel wilt has changed the composition of forests throughout the southeastern United States [26]. It has eliminated redbay and swamp bay, two keystone species, from major portions of the region, and other important species, such as sassafras, will be increasingly impacted as the disease spreads. Other rare susceptibles, such as pondberry, are threatened with extinction [24].

Time will tell when and whether new areas are affected by this destructive tree disease [28]. The ultimate impact of laurel wilt will be determined by which areas are invaded, the susceptibility of hosts that occur in these areas, the suite of vectors that are involved in new outbreaks and their effectiveness in transmitting *R. lauricola*. Clearly, ecosystems in the western United States and tropical America that are populated by susceptibles are at risk [26,28].

Laurel wilt has begun to cause alarming losses in avocado production in Florida [28]. The state's primary commercial production area was first affected in 2012, and losses will continue to increase as the disease consolidates and spreads in the area. The economic impact of laurel wilt could increase dramatically when and if it spreads to California, Mexico and other major production areas.

Much remains to be learned about this enigmatic disease. Basic information is lacking on vector x host x pathogen interactions, vector identity and ecology in the avocado system and the disease's epidemiology in natural and agricultural environments. More data are needed on the disease's host range and the nature of resistance and susceptibility. Until better information is available in these and other areas, laurel wilt will remain a destructive disease for which we have few management options.

12. Conclusions

Laurel wilt is caused by the only known systemic and lethal ambrosia beetle symbiont, *R. lauricola*. To date, all susceptibles are naïve trees in the Lauraceae plant family that do not have an evolutionary history with this pathogenic symbiont. In little more than a decade, laurel wilt spread throughout the southeastern coastal plant of the United States. Significant populations of native trees have been eliminated by the disease, and an important fruit crop, avocado, is affected in southern Florida and threatened in other production areas. Advances have been made in understanding the interactions of the pathogen with various host trees, as well as how the host responds to the disease. The disease's epidemiology is generally understood, but important gaps remain in what is known about the ambrosia beetle vector portion of the puzzle, especially in the avocado system. Although progress has been made in the management of laurel wilt, successful control in avocado production is still difficult, and in natural systems is all but impossible. An increasing impact of laurel wilt is predicted as it spreads within and beyond its present range.

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