

**Recovery Plan for**  
**Laurel Wilt on Redbay and Other Forest Species**  
**Caused by *Raffaelea lauricola* and disseminated by *Xyleborus glabratus***

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Table of Contents

Executive Summary.....	2
Contributors and Reviewers.....	4
I. Introduction.....	5
II. Disease Cycle and Symptom Development.....	9
III. Spread.....	23
IV. Monitoring and Detection.....	25
V. Response.....	29
VI. Permits and Regulatory Issues.....	32
VII. Cultural, Economic and Ecological Impacts.....	33
VIII. Mitigation and Disease Management.....	38
IX. Infrastructure and Experts.....	45
X. Research, Extension, and Education Needs.....	48
XI. References.....	50
XII. Web Resources.....	58

This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication

networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

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

### **Executive Summary**

Laurel wilt is a highly destructive disease of members of the Lauraceae in the United States. The insect vector, the redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff) was first captured in monitoring traps near Port Wentworth, GA in 2002 and first reported associated with mortality of redbay (*Persea borbonia* [L.] Spreng.) trees in 2003. Laurel wilt disease is initiated when *X. glabratus* introduces its fungal symbiont (*Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva) into the sapwood of host trees. The fungus is carried within specialized pouches in the beetle's mouthparts (mandibular mycangia), where it lives in a budding, yeast-like state. The fungal spores are introduced into the xylem as the beetle bores into the stem, leaving typical evidence of ambrosia beetle attack (small holes and boring dust). Host trees react to the fungal invasion with the production of gums and tyloses, which block water transport and cause crown wilt. Upon dissection of infected wood, xylem discoloration is readily evident.

Laurel wilt has now been detected in seven southeastern states (AL, FL, GA, LA, MS, NC, SC), causing significant mortality to redbay populations. Redbay serves an important ecological role in forests, and the loss of this species has had significant effects on forest composition. Several other

lauraceous hosts (sassafras, silk bay, swamp bay, pondspice [state endangered] and pondberry [federally endangered]) are susceptible to laurel wilt and have been affected by the disease to varying degrees. In addition, agricultural (avocado) and ornamental non-native members of the Lauraceae within the United States are susceptible, demonstrating that laurel wilt is more than a disease of native forests.

Eradication of the vector and pathogen is improbable due to the ability of the vector to persist in small diameter stems and single females to establish new populations. Currently, management options within a natural forest setting are limited and spread of the disease into new areas (e.g. California, Mexico, and Central and South America) remains a threat. For these reasons it is essential to continue monitoring the spread of the disease, and continue to develop a better understanding of the biology of the beetle and pathogen as well as the epidemiology of the disease. In addition, further development of the following strategies may help to reduce the impact of laurel wilt in forests and urban settings, and limit the spread of the disease:

- Minimization of human-aided transport of firewood and unprocessed wood materials, a mechanism for long-distance movement of the disease,
- Utilization of chemical options (fungicides and possibly insecticides) for the protection of high value trees,
- Continued development of resistant host plant cultivars for landscape use and restoration,
- Collection and maintenance of germplasm of vulnerable hosts, especially rare species that may be in danger of extinction,
- Continued research on disease biology, vector chemical ecology, alternative disease pathways and vectors, management options, and natural enemies, and
- Continued efforts to educate the public about the potential cultural, economic and ecological effects of laurel wilt.

## Recovery Plan for

### Laurel Wilt on Redbay and Other Forest Species

Caused by *Raffaelea lauricola* and disseminated by *Xyleborus glabratus*

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## I. Introduction

Laurel wilt is a disease of shrubs and trees within the plant family Lauraceae. The fungal pathogen (*Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva) is transmitted into the host xylem by the redbay ambrosia beetle (*Xyleborus glabratus* Eichhoff) when the beetle bores into the trunk or large stems (Harrington et al. 2008). *Xyleborus glabratus* and *R. lauricola* are both exotic organisms in the USA, which were presumably transported within solid wood packing material prior to the beetle's first detection in Port Wentworth, GA in 2002 (Fraedrich et al. 2008). Laurel wilt has caused a highly significant reduction in redbay (*Persea borbonia* [L.] Spreng.) populations within forests of the southeastern United States, with mortality of redbay and other host species recorded in seven states (AL, FL, GA, LA, MS, NC, SC) ([http://www.fs.fed.us/r8/foresthealth/laurelwilt/dist\\_map.shtml](http://www.fs.fed.us/r8/foresthealth/laurelwilt/dist_map.shtml)). Within weeks to months of inoculation by the beetle, infected redbay trees will display wilt in a portion of the canopy that subsequently extends to the entire crown; upon death the trees become suitable substrates for reproduction of the redbay ambrosia beetle (Fraedrich et al. 2008).

The redbay ambrosia beetle is native to southern Asia (India, Bangladesh, Myanmar, Japan, Taiwan and China) (Rabaglia et al. 2006, Hulcr and Lou 2013). *Raffaelea lauricola* has been isolated from beetles from Japan and Taiwan, suggesting that their symbiotic relationship occurs within their native range (Harrington et al. 2011). In the United States *X. glabratus* has only been recorded in plants within the Lauraceae; although *R. lauricola* has also been recovered from additional ambrosia beetle species (Carrillo et al. 2014) and also live oak (*Quercus virginiana*) (J. Smith, personal communication). Specimen records suggest that *X. glabratus* also strongly prefers lauraceous hosts in its native range, although it has also been collected from species in the Dipterocarpaceae, Fagaceae, Fabaceae, Theaceae and Pinaceae (Rabaglia et al. 2006, Hulcr and Lou 2013). To the authors' knowledge, there are no reported cases of laurel wilt outside the United States.

Laurel wilt has naturally affected most native and several cultivated nonnative species of the Lauraceae within the southeastern United States. *Raffaelea lauricola* has been recovered from symptomatic forest and landscape (indicated with an asterisk) plants and Koch's postulates completed for:

○ Redbay	<i>Persea borbonia</i> (L.) Spreng.	[Fraedrich et al. 2008]
○ Swamp bay	<i>Persea palustris</i> (Raf.) Sarg.	[Fraedrich et al. 2008]
○ Sassafras	<i>Sassafras albidum</i> (Nutt.) Nees	[Fraedrich et al. 2008]
○ Avocado*	<i>Persea americana</i> Mill.	[Mayfield et al. 2008a]
○ Pondspice <sup>a</sup>	<i>Litsea aestivalis</i> (L.) Fernald	[Hughes et al. 2011]
○ Pondberry <sup>a</sup>	<i>Lindera melissifolia</i> (Walter) Blume	[Fraedrich et al. 2011]
○ Silk bay	<i>Persea humilis</i> Nash	[Hughes et al. 2012]
○ Bay laurel*	<i>Laurus nobilis</i> (L.)	[Hughes et al. 2014]
○ Camphortree*	<i>Cinnamomum camphora</i> (L.) J. Presl	[Fraedrich et al. in press]

The following hosts have not been infected in the landscape; however, laurel wilt symptom development has occurred after artificial inoculation with *R. lauricola*:

○ California laurel	<i>Umbellularia californica</i> (Hook. & Arn.) Nutt.	[Fraedrich 2008]
○ Northern spicebush	<i>Lindera benzoin</i> (L.) Blume	[Fraedrich et al. 2008]
○ Gulf licaria <sup>a</sup>	<i>Licaria triandra</i> (Sw.) Kosterm.	[Ploetz and Konkol 2013]
○ Viñátigo (Spanish)	<i>Persea indica</i> (L.) Spreng.	[Hughes et al. 2013]
○ Lancewood	<i>Ocotea coriacea</i> (Sw.) Britton	[Hughes and Ploetz unpublished]

<sup>a</sup> = indicates threatened or endangered status (state or federally)

Redbay is an aromatic evergreen tree with leathery leaves that often forms a dense and rounded crown. Redbay, swamp bay and silk bay are often grouped together as a single species called "redbay"; however, differences exist in morphology, canopy architecture, and habitat (Coder 2007), as well as in secondary metabolites (Niogret et al. 2011). Redbay (*sensu stricto*) is the species most affected by laurel wilt. The disease spreads rapidly within stands and typically over 90% of the redbay trees in an area die within a few years (Fraedrich et al. 2008, Shield et al. 2011, Cameron et al. 2012,

Evans et al. 2013, Spiegel and Leege 2013). In newly infested sites, *X. glabratus* will preferentially attack large diameter trees but also infests smaller trees, with most attacks concentrating on the lower bole (Fraedrich et al. 2008, Maner et al. 2012, Kendra et al. 2013a, Mayfield and Brownie 2013). Dispersing beetles are attracted to host volatiles (Hanula and Sullivan 2008, Kendra et al. 2011 & 2012b, Niogret et al. 2011, Kuhns et al. 2014a) and also use stem silhouettes as visual cues (Mayfield and Brownie 2013) when finding host trees.

Laurel wilt of avocado was first observed in residential areas near Jacksonville, FL in 2007 (Mayfield et al. 2008a), and in 2012 was first observed in the avocado production areas of Miami-Dade County (Ploetz et al. 2013). A separate recovery plan for laurel wilt in avocado was prepared (Ploetz et al. 2011a), and is being revised.

The development of laurel wilt in sassafras is slightly different than in redbay, perhaps because sassafras is deciduous and can propagate from interconnected root sprouts. Leaves of affected sassafras display a green to brown transition followed by wilt and defoliation (Fig. 1), while in redbay the wilted leaves often remain attached for long periods of time. If infected in early spring leaf expansion may cease, resulting in stunted foliage, followed by wilting and defoliation. The presence of dark vascular discoloration in lateral roots of infected sassafras (Fig. 1C), along with the directional movement of laurel wilt among thickets without evidence of *X. glabratus* attack suggests underground transmission of the pathogen through roots and stolons (Cameron et al. 2010 & 2012).

Laurel wilt has caused mortality to the federally endangered pondberry (GA and SC) and state threatened/endangered pondspice (GA, SC, FL), with *X. glabratus* brood production confirmed within pondspice (Fraedrich et al. 2011, Hughes et al. 2011). Although these shrubs are considered suboptimal hosts for *X. glabratus*, due to their small diameter stems (Fraedrich et al. 2011), their ultimate fate remains uncertain.

Branch dieback due to laurel wilt was confirmed in camphortree in FL and GA; however, mortality in this Asian species has been limited (Cameron et al. 2008, Smith et al. 2009). *R. lauricola* moved systemically in the xylem of camphortree after artificial inoculation, and multiple stem infections caused mortality in saplings (Fraedrich et al., in press [<http://onlinelibrary.wiley.com/doi/10.1111/efp.12124/pdf>]). Presumably, this species co-evolved with the beetle and fungus in Asia and therefore has more tolerance than the non-co-evolved North American hosts.

A single bay laurel (*Laurus nobilis*) located near a recently wilted avocado in Gainesville, FL also succumbed to laurel wilt in 2013 (Hughes et al. 2014). Bay laurel is a small to moderate sized tree that is native to areas of the southern Mediterranean, but it is planted in residential areas as an ornamental in the United States because of its attractive form and culinary use of leaves.

In order to predict future host range expansions, inoculation experiments with other lauraceous plants have been conducted. Northern spicebush, lancewood, and Gulf licaria are species within the current geographic range of laurel wilt that have shown varying levels of symptom development following artificial inoculation. Gulf licaria is a federally endangered species, with a natural population of less than 12 trees found within Miami-Dade County (Surdick and Jenkins 2010, Ploetz and Konkol 2013), thus any mortality of this species would be ecologically significant. Potted California laurel (*Umbellularia californica*) seedlings exhibited susceptibility to *R. lauricola*, and bolts from trees attracted *X. glabratus* in field experiments and supported vector reproduction. This suggests that *U. californica* may be a viable host should *X. glabratus* invade the western U.S. (Fraedrich 2008, Mayfield et al. 2013). *Persea indica*, a dominant member of the fragile laurel cloud forests of the Madeira and Canary Islands, and used as an ornamental in areas of the USA and Spain with Mediterranean-like climates, was also reported to be attractive to *X. glabratus* and susceptible to *R. lauricola* (Peña et al. 2012, Hughes et al. 2013). The attractiveness of *L. nobilis* and *P. indica* to *X. glabratus* and their susceptibility to *R. lauricola*



suggests that an importation event to their natural ranges could lead to laurel wilt outside the USA. The establishment of laurel wilt in new regions under natural conditions may be more difficult than suggested by artificial experimentation and is contingent on a number of factors including: 1) the arrival of *X. glabratus* to these new locations (naturally or human assisted), 2) the ability for *X. glabratus* to locate susceptible hosts, and 3) the establishment of brood and successful reproduction within new host material.

## II. Disease Cycle and Symptom Development

The disease cycle begins as female redbay ambrosia beetles disperse during late afternoon and early evening (Brar et al. 2012, Kendra et al. 2012a) in search of a viable host (Fig. 2). Guided by host volatiles and visual silhouettes, the female *X. glabratus* will land on and bore into the trunk and large stems of redbays (majority of boring holes occur below a stem height of 1.5 m) and other members of the plant family Lauraceae (Fraedrich et al. 2008, Hanula et al. 2008, Niogret et al. 2011, Kendra et al. 2011, 2012a, 2013a, 2014a, Brar et al. 2012, Maner et al. 2012, Mayfield and Brownie 2013, Kuhns et al. 2014a). During its boring attempts *X. glabratus* deposits *R. lauricola* from specialized conidia-bearing pouches (mycangia) near its mandibles, into the sapwood, thus inoculating the tree. Spores of *R. lauricola* migrate passively through the xylem, causing the tree to produce gums and tyloses which impede water transport and cause the foliage to wilt (Inch and Ploetz 2012, Inch et al. 2012). Wilt can occur within a few weeks of inoculation, with preliminary symptoms appearing as dark olive green, reddened, or browning leaves and drooping foliage in localized portions of the crown (Fig 3A). As symptoms progress, the wilt spreads to the rest of the canopy, resulting in complete crown wilt with marcescent brown leaves (Fig 3B,C). Within infected trees, removal of the bark will reveal xylem discoloration (brown/black streaking along the vessels) (Fig. 4). Disease progression from initial inoculation to complete crown wilt and tree death may take a few weeks to months, depending on

environmental conditions. Affected trees become more attractive to mass attacks from *X. glabratus* and other ambrosia beetle species as symptoms develop. It is possible that plant-fungal interactions increase release of volatiles attractive to *X. glabratus* females and that of other ambrosia beetle species. Also, the symbiotic fungi of some ambrosia beetles in Florida emit volatiles that attract their specific beetle species (Hulcr et al. 2011, Kuhns et al. 2014b). Root graft transmission of *R. lauricola* is a possibility, especially in hosts with connected root systems; however, scientific studies have yet to confirm this avenue of spread. Root graft transmission appears to be contributing significantly to the local spread of laurel wilt in avocado groves (R. C. Ploetz, personal communication).

Evidence of ambrosia beetle attacks can be seen by the appearance of ephemeral tubes of boring dust (“frass tubes”) that will hang from the trunk of attacked trees (Fig. 5A). Boring dust tubes are very delicate, and will eventually collapse due to wind or rain, leaving an accumulation of boring dust on the lower trunk or base of the tree (Fig. 5B). Females of *X. glabratus* lay eggs in the natal galleries in the xylem (Figs. 6 & 7A), and developing larvae and adults likely feed on *R. lauricola* (and other symbionts) that have colonized the gallery walls (Fig. 7B).

*Xyleborus glabratus* emergence can begin as early as 40 days after gallery formation in summer months (development is slower in colder months), with overlapping generations often occurring within the same tree (Hanula et al. 2008, Brar et al. 2013, Maner et al. 2013). Once mature, *X. glabratus* females emerge from their gallery systems and locate new hosts for the development of subsequent generations. Infested redbays can remain standing and galleries can remain active for over a year (Brar et al. 2013, Maner et al. 2013). *Raffaelea lauricola* can still be recovered from trees for over a year after mortality until other decay consumes the tree, leading to eventual breakage of the main stem (Spence et al. 2013) (Fig. 8).

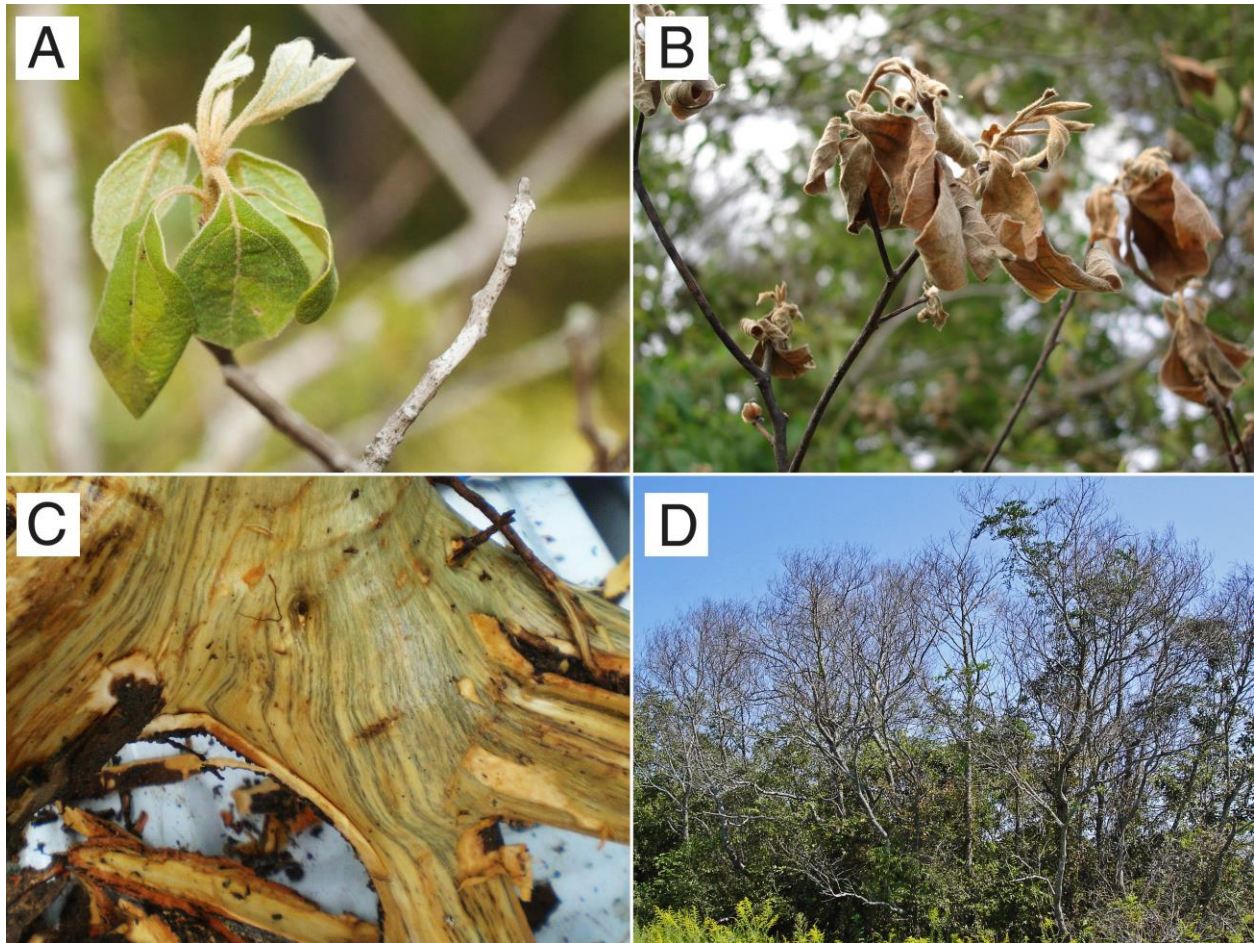
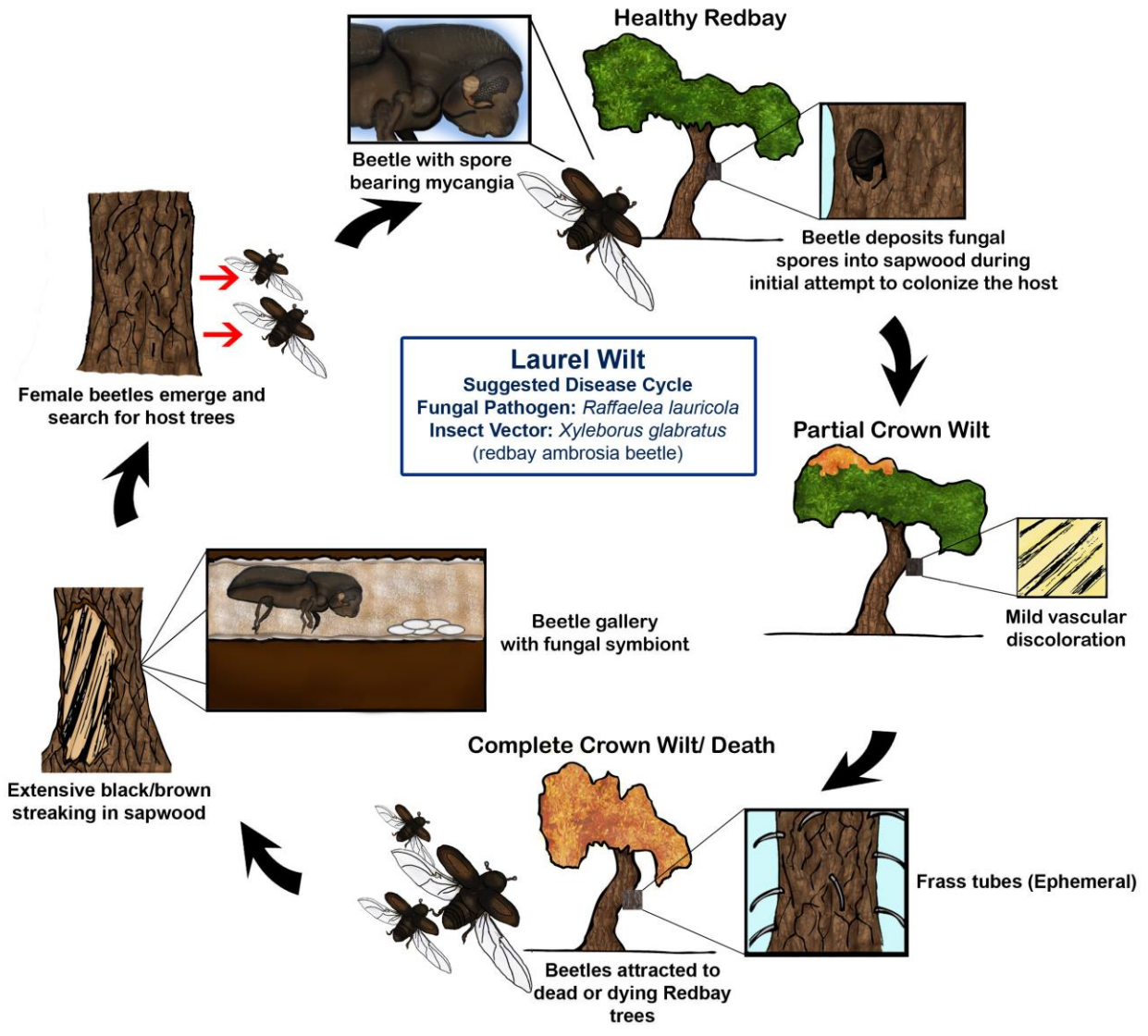


Figure 1. Laurel wilt development in sassafras. A) Green, wilted foliage, that progresses to B) Brown wilted foliage, C) Sapwood discoloration on root flare, D) Standing, defoliated trees. Photos by Scott Cameron (A, B, D) and Chip Bates (C) – Georgia Forestry Commission.



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Figure 2. Laurel wilt disease cycle in redbay

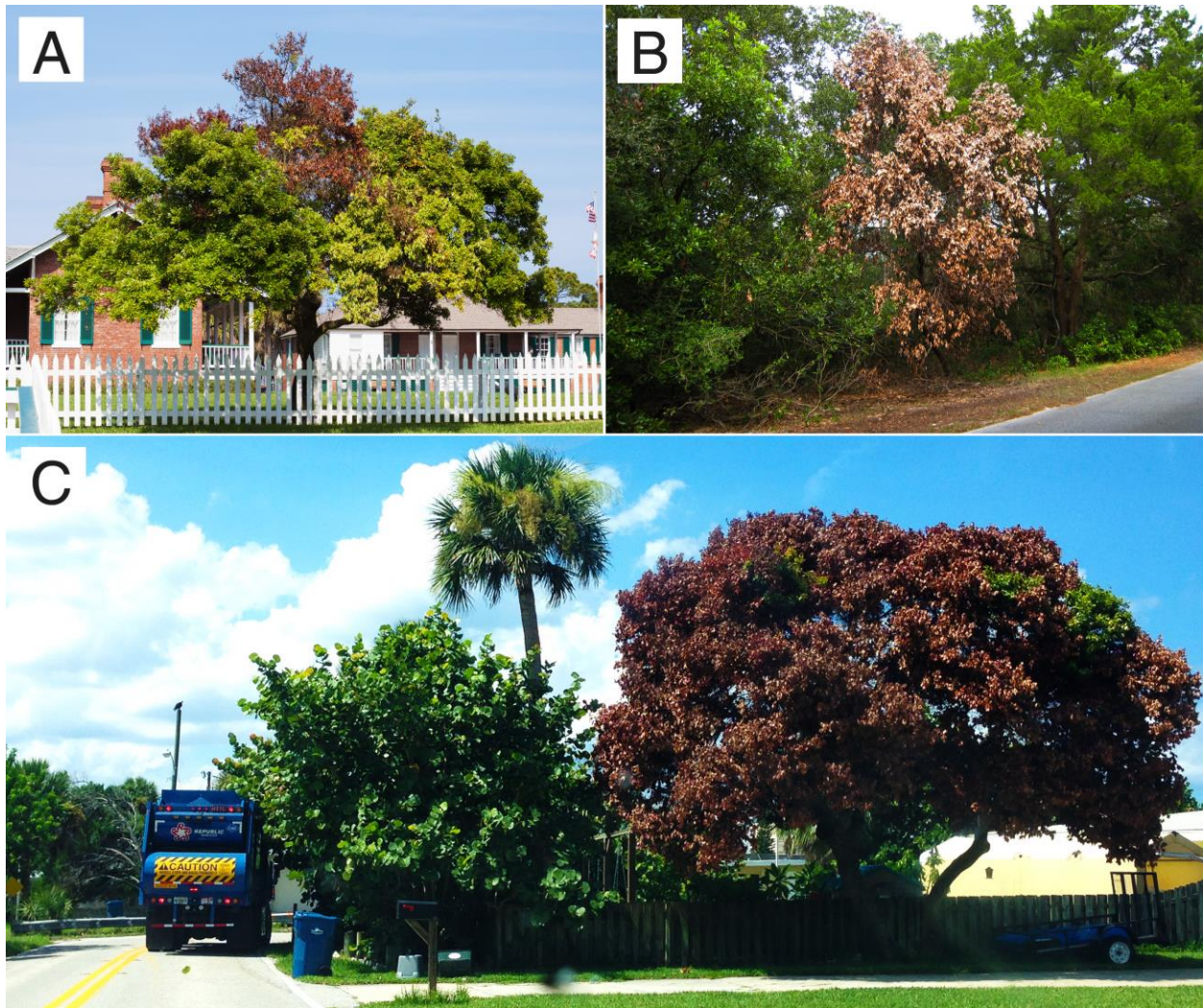


Figure 3. Laurel wilt external symptom development on redbay. A) browning of foliage and wilt of localized portions of the upper crown, B and C) complete crown wilt with attached leaves. Photos by Marc A. Hughes- University of Florida

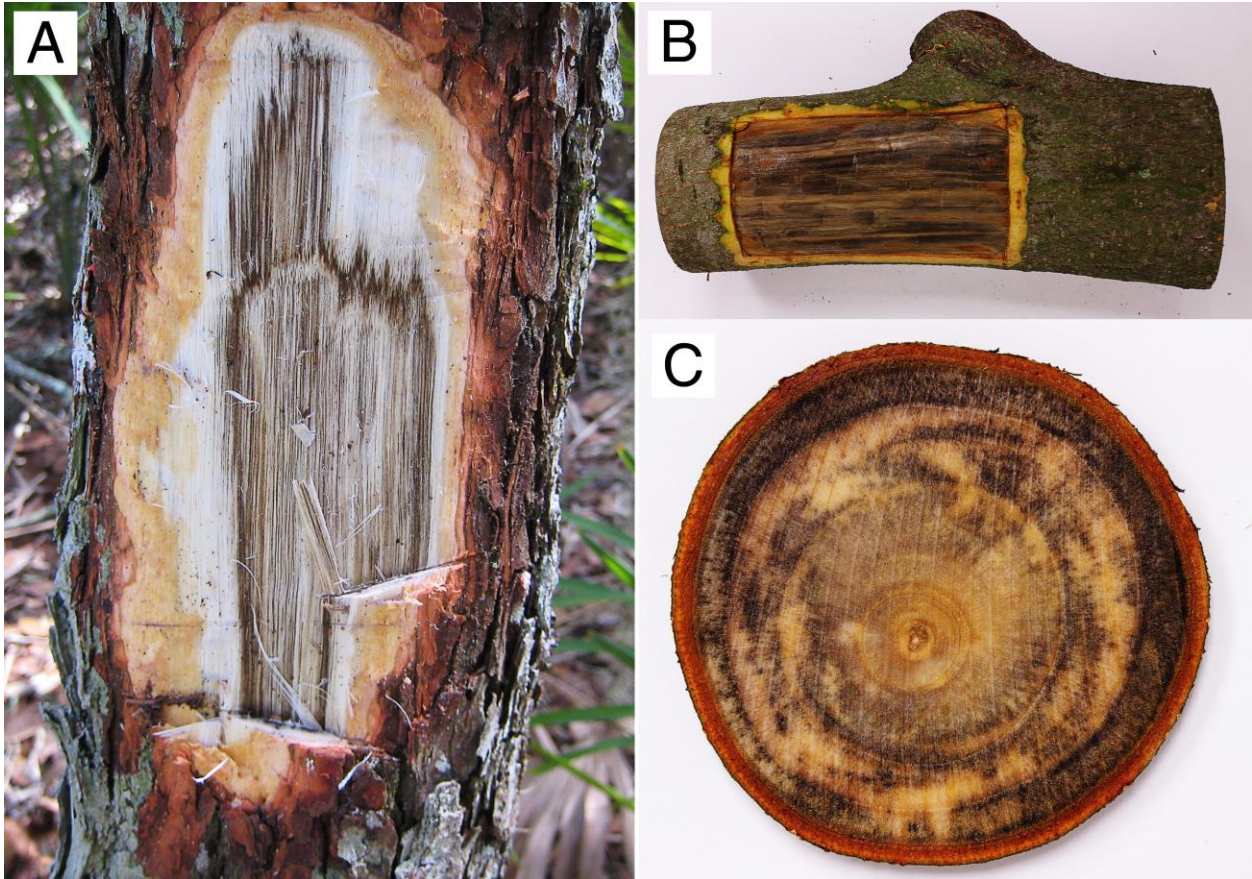


Figure 4. Sapwood discoloration in laurel wilt-affected redbay trees. A and B) Longitudinal, and C) Transverse sections. Photos by Marc A. Hughes-University of Florida

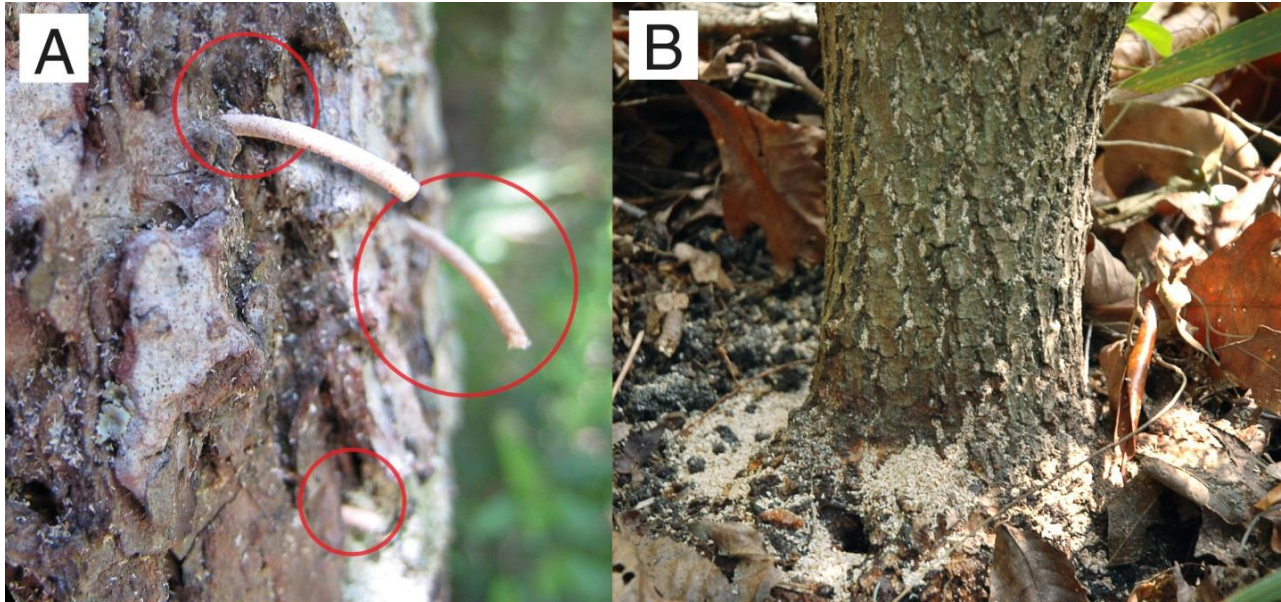


Figure 5. Evidence of ambrosia beetle boring activity. A) Tubes of boring dust indicating recent entrance of ambrosia beetles into the trunk or stem. B) Boring dust at the base of redbay tree indicating mass attack. Due to their delicate nature, dust tubes or the piles of dust at the base of trees can be carried away with rain or wind. Boring dust is a general symptom of any ambrosia beetle species attack and does not always indicate *X. glabratus*. Photos by Marc A. Hughes-University of Florida.



Figure 6. Redbay ambrosia beetle (*Xyleborus glabratus*) natal gallery in redbay with adult beetle and eggs. Photo by Lyle J. Buss-University of Florida.

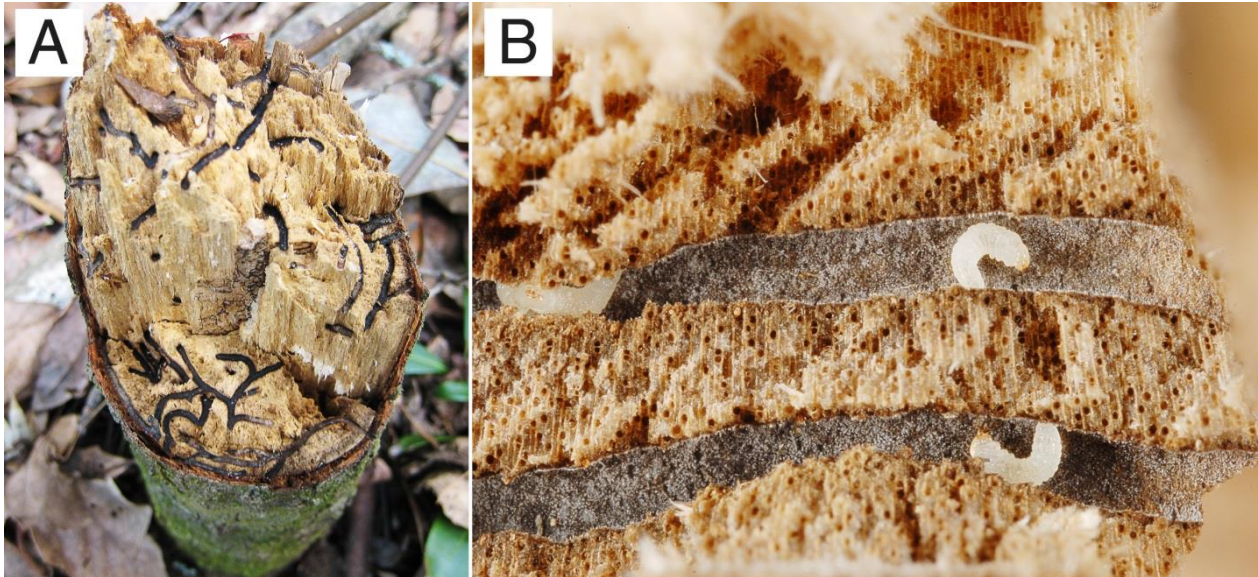


Figure 7. Redbay ambrosia beetle (*Xyleborus glabratus*) natal galleries in redbay tree. A) Extensive gallery systems in transverse section. B) *X. glabratus* larvae feeding on symbiotic fungi that line gallery walls (white fuzzy material). Photos by Marc A. Hughes (A) and Lyle J. Buss (B) - University of Florida.

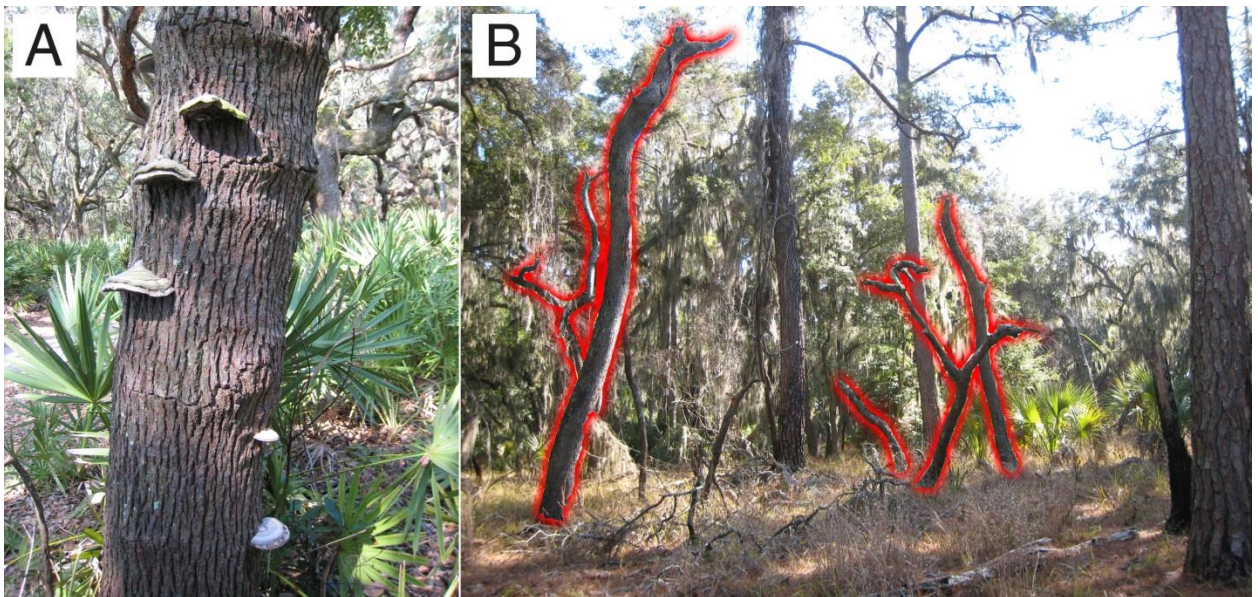


Figure 8. Fate of redbay trees after laurel wilt mortality. A) Dead redbay tree colonized with decay fungi (in this case, *Fomes fomentarius*). B) Breakage of redbay trunks (outlined in red) due to the loss of structural integrity from decay. Photos by Marc A. Hughes- University of Florida.



*Xyleborus glabratus* has a haplo-diploid mating system, in which males develop from unfertilized eggs and possess only a single set of chromosomes (haploid), while the females (like most other animals) have both their mother's and father's genes (diploid). Males are rare compared to females (Maner et al. 2013 reported a 27:1 female to male ratio), are flightless, lack mycangia, and stay near or within the natal gallery for inbreeding with their sisters to produce more females (Maner et al. 2013). *Xyleborus glabratus* females, with their ability to fly and transmit *R. lauricola* are the main drivers in the laurel wilt epidemic.

Studies on the host seeking behavior of *X. glabratus* show that females are attracted to volatile terpenoids produced by host trees, including  $\alpha$ -copaene,  $\alpha$ -cubebene,  $\alpha$ -humulene, calamenene and eucalyptol (Hanula and Sullivan 2008, Kendra et al. 2011, 2014a, Kuhns et al. 2014a), but not to ethanol, which is attractive to most other ambrosia beetle species (Hanula and Sullivan 2008, Kendra et al. 2014b). In sites new to laurel wilt infestation, large diameter trees may be attacked first, indicating a preference of *X. glabratus* for larger stems; however, during an outbreak all size classes can be affected simultaneously (Fraedrich et al. 2008, Kendra et al. 2013a, Mayfield and Brownie 2013, J. Eickwort, personal observation). Observations also suggest that the first trees affected within a redbay stand may often be stressed (damage or due to other factors) (S. Cameron, personal observation). In the absence of large redbays, *X. glabratus* populations can persist on small diameter stems at low levels (Maner et al. 2014).

*Xyleborus glabratus* females (Fig. 9A, B) are slender and cylindrical (2.1 - 2.4 mm long), and similar in size and shape to several other ambrosia beetle species. Characteristics useful for identifying adult females include their size which is smaller than most native ambrosia beetles, dark brown to black color, nearly glabrous (shiny) upper elytral surface with very little to no vestiture (hairs), and the distinctly angular elytral declivity. Males (Fig. 9C, D) are similar to females but are smaller (1.8 mm

long), with a pronotum that is anteriorly flattened and has two, short flattened spines extending over the head capsule (Rabaglia et al. 2006).



Figure 9. Redbay ambrosia beetle (*Xyleborus glabratus*). A) Dorsal view of adult female, B) Lateral view of adult female, C) Dorsal view of adult male, D) lateral view of adult male. Photos by Lyle J. Buss-University of Florida.

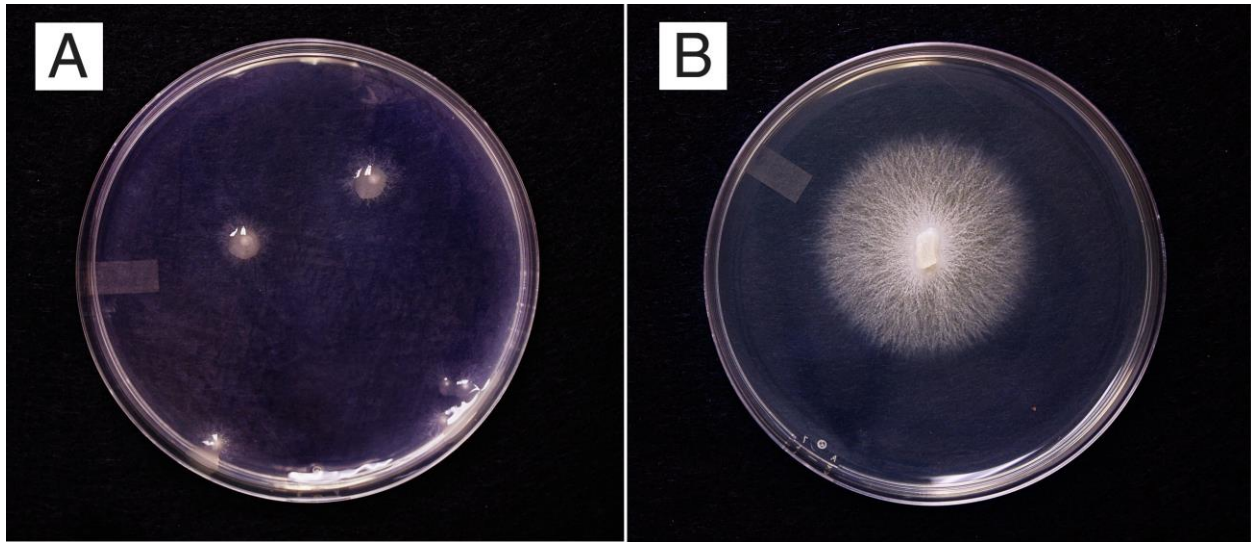


Figure 10. *Raffaelea lauricola* on malt extract agar as a, A) Yeast phase (5d old), and B) filamentous form (10d old). Photos by Marc A. Hughes-University of Florida.

*Raffaelea lauricola* is an asexual, di-morphic fungus that produces a yeast phase (budding cells) in the mycangium of *X. glabratus*, culture media (Fig. 10A) and in plant hosts, and mycelium on gallery walls and culture media (Fig. 10B). Within galleries, the fungus produces tightly packed conidiophores (sporodochia) that are grazed on by the developing larvae and adults (Harrington et al. 2008). Up to six *Raffaelea* species have been isolated from *X. glabratus*; however, the role of species other than *R. lauricola* is unclear (Harrington et al. 2010). Their pathogenicity has been tested in swamp bay, but none other than *R. lauricola* have led to laurel wilt symptoms (T. Dreaden, personal communication). *Raffaelea lauricola* is the primary and most abundant symbiont of *X. glabratus* with most beetle individuals harboring hundreds to thousands of spores within their mycangia (Harrington and Fraedrich 2010, Harrington et al. 2011, Carrillo et al. 2014). The fact that a dose of only 100 spores is able to cause wilt in swamp bay and avocado indicates that *R. lauricola* is highly virulent (Hughes et al. in press. [<http://onlinelibrary.wiley.com/doi/10.1111/efp.12134/pdf>]).

*Raffaelea lauricola* has been isolated from other ambrosia beetle species (*Xyleborinus saxesenii* Ratzeburg, *Xylosandrus crassiusculus* Motschulsky, *Xyleborus ferrugineus* Fabricius, *Xyleborus affinis*

Eichhoff, *Xyleborus volvulus* Fabricius, *Xyleborus bispinatus* Eichhoff, and *Xyleborinus gracilis* Eichhoff) (Harrington and Fraedrich 2010, Harrington et al. 2011, Carrillo et al. 2014), but it is unclear whether these species can transmit the pathogen in nature and cause disease in otherwise healthy trees. Carrillo et al. (2014) documented that six ambrosia beetle species other than *X. glabratus* (above in **bold**) reared from laurel wilt affected logs harbored *R. lauricola*. The pathogen was isolated from cohorts of each species that were then used to challenge redbay and avocado in no-choice tests. *Raffaelea lauricola* was recovered in avocado challenged with *X. volvulus* and *X. ferrugineus*, with *X. volvulus* able to induce laurel wilt, while to varying degrees, all species were able to transmit *R. lauricola* and cause laurel wilt in redbay. The probability of a beetle carrying *R. lauricola* was significantly lower in ambrosia beetles that acquired the pathogen via lateral transfer, thus a high attack density (40 beetles) was used in the no-choice tests (Carrillo et al. 2014). In general, the beetles found to carry *R. lauricola* did so at much lower levels (1-100 colony forming units [CFU] per beetle, depending on species) vs. *X. glabratus* ( $\approx$  2800 CFU per beetle) (Carrillo et al. 2014). More research is needed to understand the role that other species of ambrosia beetles could play in the epidemiology of laurel wilt.

The black twig borer (*Xylosandrus compactus* Eichhoff) is another ambrosia beetle whose damage can be misdiagnosed as the early stages of laurel wilt. The black twig borer (Fig. 11A) attacks small stems (typically less than 5 cm in diameter) on redbay, camphortree, as well as many other tree species throughout the Southeastern U.S. (Dixon et al. 2011, Fraedrich et al. in press). Damage by this beetle appears as the browning and wilting of individual branches (flagging) or stems (Fig. 11B). Attacks appear as a single pinhole on the stem (Fig. 11C), where the female constructs and lays eggs within the pith (Fig. 11D). The stem is hollowed and surrounding xylem exhibits dark discoloration that will remain localized to within a few centimeters of the brood cavity (Dixon et al. 2011). Several differences exist between black twig borer damage and laurel wilt including: 1) branch and twig wilt associated with black twig borer is usually less severe and often scattered throughout the crown with intermittent green

healthy foliage, whereas laurel wilt initially affects larger crown sections but within days will often affect the entire crown, 2) attacks on small diameter stems with hollowed pith are suggestive of black twig borer attack, while the majority of the redbay ambrosia beetles' attacks and boring will be on the lower trunk and large stems of the host, and 3) vascular discoloration of the black twig borer will remain only on infected twigs and branches in close proximity to their entrance holes, while the xylem discoloration associated with laurel wilt is systemic and can be observed over large portions of the main stem and eventually the entire trunk of the tree. Due to their overlapping geographic and host ranges, one frequently observes damage by both *X. glabratus* and *X. compactus*; however, only laurel wilt is lethal to mature trees.

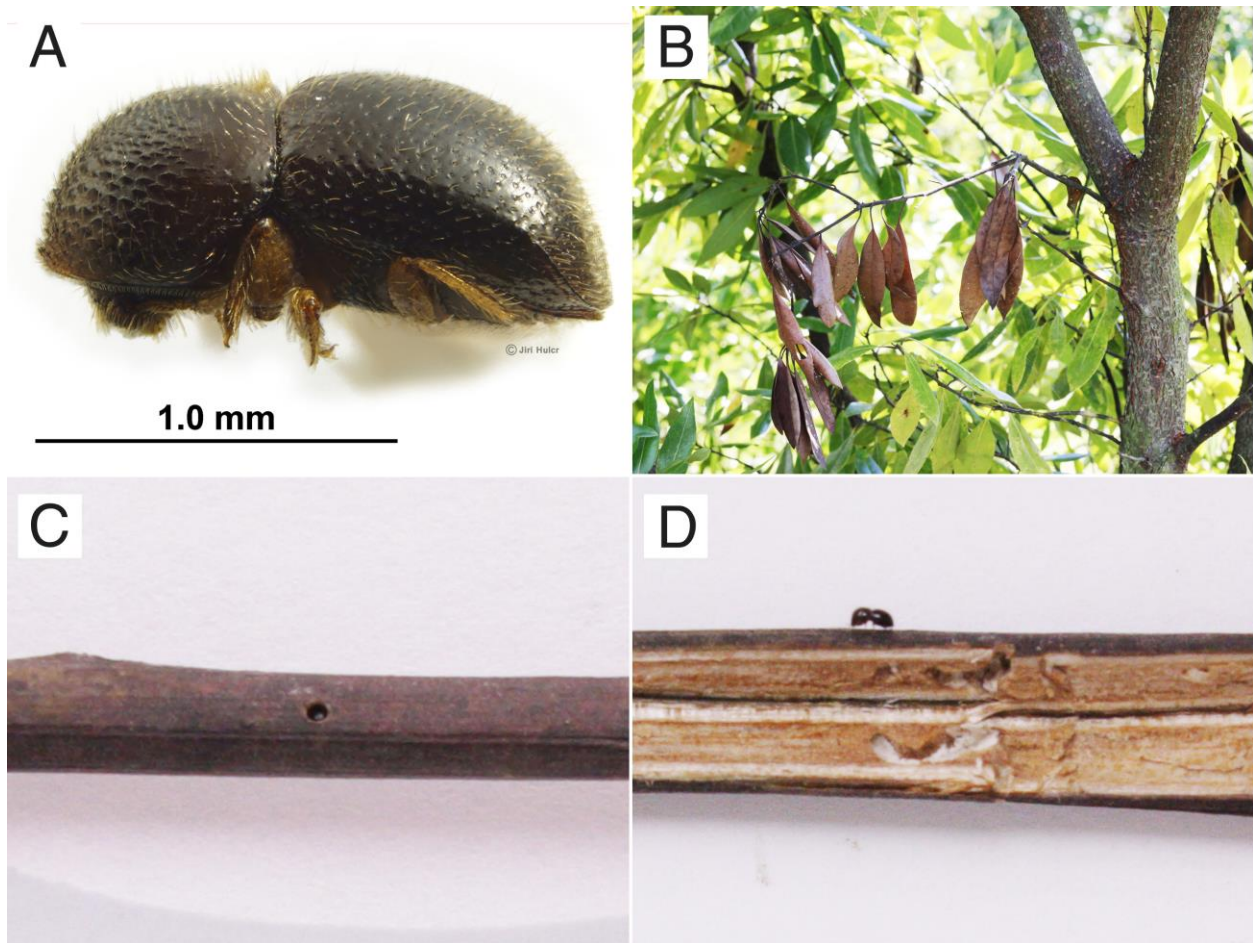


Figure 11. Black twig borer (*Xylosandrus compactus*). A) Lateral view of adult female, B) Twig wilt (flagging) on redbay, C) Entrance hole, and D) Longitudinal section of affected twig showing a gallery within the pith of redbay and *X. compactus*. Photos by Jiri Hulcr (A) and Marc A. Hughes (B, C, D) - University of Florida.

### III. Spread

Distribution of Counties with Laurel Wilt Disease\* by year of Initial Detection

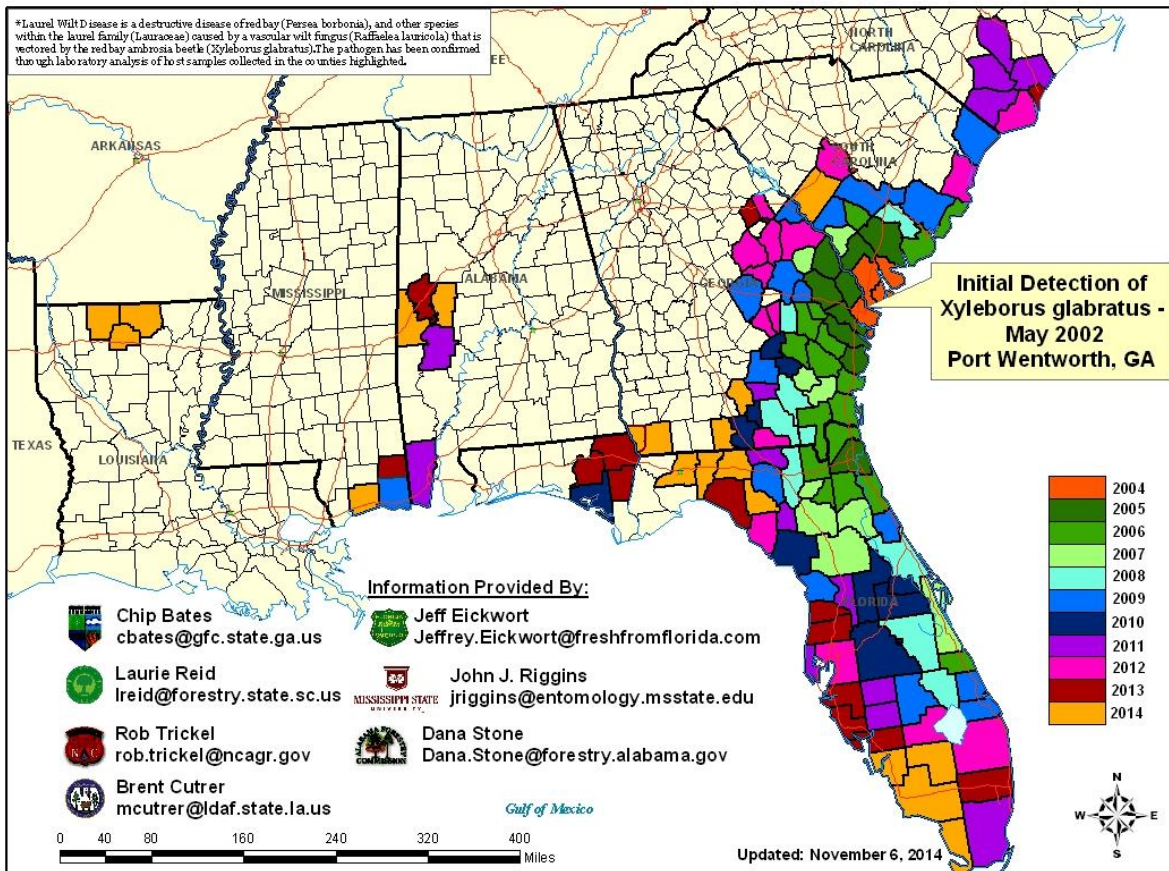


Figure 12. Laurel wilt distribution map. Color within a county indicates first year of confirmed laurel wilt infection. Available on: [http://www.fs.fed.us/r8/foresthealth/laurelwilt/dist\\_map.shtml](http://www.fs.fed.us/r8/foresthealth/laurelwilt/dist_map.shtml)

*Xyleborus glabratus* was initially detected in early monitoring traps near Port Wentworth GA in 2002 (Haack 2006). Laurel wilt was first recognized in Chatham County GA and in Beaufort and Jasper Counties SC in 2004. The disease has radiated outward along the Atlantic Coastal Plain and after 10 years has spread to 7 states (AL, FL, GA, LA, MS, NC, SC) (Fig. 12). Movement of the disease has mostly occurred to adjacent counties, but discontinuous jumps in distribution have occurred (Fig. 12). The movement of laurel wilt to Volusia County, FL in 2008 was caused by the transport of infected logs by a resident for woodturning purposes (A. E. Mayfield, personal communication). The infestation of an area

adjacent to a mulch plant in Brantley County GA (2007), which was surrounded by unaffected counties also suggests disease spread via movement of infected wood material (Cameron et al. 2008). Other geographic jumps in distribution appear to have occurred in Mississippi (2009), the Florida panhandle (2010), Alabama (2011), and Louisiana (2014) suggesting that the movement of infested wood products to new areas has occurred several times.

Little is known about the distance *X. glabratus* travels in dispersal flights. The mapping of laurel wilt spread among the Florida Everglades' tree islands revealed a range of 1.0 -11.8 km between neighboring symptomatic islands (Rodgers et al. 2014), suggesting that some long distance flight (with the potential aid of wind currents) is possible, with an estimated spread rate up to 55 km per year (Koch and Smith, 2008). Climate matching models generated by Koch and Smith (2008) predicted that the movement of laurel wilt would closely follow the documented geographic range of redbay (Coastal plain of the Atlantic and Gulf coasts), with the assumption that sassafras would not serve as a host and climatic constraints would limit its northern spread. However, laurel wilt disease and *X. glabratus* has been documented in sassafras stands in Georgia, Mississippi and Marengo County Alabama, where redbay does not exist (Riggins et al. 2011, Cameron et al. 2012, Bates et al. 2013). In addition, studies examining cold tolerance (supercooling point) showed that *X. glabratus* was able to withstand temperatures as low as -24°C, thus the spread model of Koch and Smith (2008) may have underestimated the ability of the beetle to move to colder northern areas where sassafras would be the main host (Formby et al. 2014).

In addition to vector spread by natural and human assisted movement, anecdotal evidence suggests that root graft transmission may be occurring within sassafras and the commercial avocado groves. Vascular discoloration of roots of affected swamp bay, avocado, and sassafras is evident, and *R. lauricola* has been isolated from roots of swamp bay and avocado (Cameron et al. 2010, Hughes et al. in press, R. C. Ploetz, personal communication). The directional spread of laurel wilt from a central focus



along rows of avocados and sassafras thickets, without obvious ambrosia beetle attacks in the neighboring trees suggests root graft transmission of *R. lauricola* (Cameron et al. 2010, R. C. Ploetz, personal communication). At this time the importance of root graft transmission is less clear in redbay and swamp bay because of the lack of clearly observable infection foci in stands with these species, and because of the lack of empirical evidence of redbay root grafting.

#### **IV. Monitoring and Detection**

##### Vector Detection

Monitoring and surveys have been conducted by state agencies with funding and additional support from federal agencies. Within Florida, updates on the movement of the disease have been accomplished by field detections and reports by the Florida Forest Service, the Division of Plant Industry, and county extension agents from the University of Florida. The Florida Cooperative Pest Survey (CAPS) has surveyed areas in Miami-Dade County in order to delineate areas of *X. glabratus* infestation by scouting host-dense natural areas and by the deployment of Lindgren funnel traps baited with manuka oil lures, with special attention to the avocado production areas (Whilby et al. 2012). Ongoing surveys by the Alabama, Georgia, South Carolina, and Mississippi Forestry Commissions, Mississippi State University, the North Carolina Forest Service, and the Louisiana Department of Agriculture and Forestry are being conducted based on vector trapping and field scouting of infected sites to estimate the impacts of laurel wilt disease (Boone 2010, Cameron et al. 2008 & 2010; R. Trickel, B. Cutrer and D. Stone, personal communications).

Early trapping attempts revealed that *X. glabratus* was not attracted to ethanol lures like most ambrosia beetle species which prefer stressed and dying trees (Hanula and Sullivan 2008, Kendra et al. 2012b). Cut bolts or logs from host trees are attractive to *X. glabratus* and can be used to lure and collect live beetles (Hanula et al. 2008, Kendra et al. 2012b). However, due to the dwindling amount of

fresh host material in areas affected by laurel wilt, this method was not feasible for long term monitoring efforts. Collection of volatiles from host tree wood (Hanula and Sullivan 2008, Niogret et al. 2011) and from the non-host yet highly attractive *Litchi chinensis* Sonn., (Kendra et al. 2011, 2013b) revealed several chemical constituents in common, predominantly the sesquiterpene  $\alpha$ -copaene. Since  $\alpha$ -copaene is expensive and difficult to synthesize, several essential oils high in  $\alpha$ -copaene were evaluated as field lures. Initial research identified manuka oil (an extract from *Leptospermum scoparium* J.R.Forst. & G.Forst.) and phoebe oil (from *Phoebe porosa* [Nees & Mart.] Mez) as attractive baits for *X. glabratus* (Hanula and Sullivan 2008). With the commercial availability of manuka oil lures, various agencies and researchers adopted this product in conjunction with a Lindgren funnel trap as the standard detection system. Unfortunately, manuka oil lures have been shown to be sub-optimal due to their: 1) limited window of attractiveness observed in field trials (as little as 2 weeks), 2) variability in the efficacy of lures manufactured in different years and 3) attraction of various non-target species (Kendra et al. 2011, 2012c, Brar et al. 2012, Hanula et al. 2013). Although phoebe lures were superior to manuka lures in attractiveness and duration (Kendra et al. 2012c), they are no longer an option due to the limited supply of source tree material. Subsequent research identified cubeb oil (from *Piper cubeba* L.) as a new attractant for *X. glabratus* (Kendra et al. 2013a), and a commercially available bubble lure containing distilled cubeb oil was shown to be more attractive than the manuka lure and lasted at least 12 weeks (Hanula et al. 2013, Kendra et al. 2014b, 2015). Future monitoring efforts should consider the use of cubeb oil lures for more sensitive detection of *X. glabratus*.

A recent comparative study (Kendra et al. 2014a) evaluated nine species within the Lauraceae in terms of terpenoid emissions and relative attraction of *X. glabratus* (Fig. 13). In addition to  $\alpha$ -copaene, three other sesquiterpenes were positively correlated with attraction:  $\alpha$ -cubebene,  $\alpha$ -humulene, and calamenene. Interestingly, California bay laurel, a species attractive to female *X. glabratus* (Mayfield et al. 2013) and susceptible to laurel wilt (Fraedrich 2008), was found to have low sesquiterpene content

and high emissions of eucalyptol, a monoterpene ether. Eucalyptol alone, in high doses, has also been shown to attract *X. glabratus* (Kuhns et al. 2014a). This information suggests that multiple chemical cues may contribute to the location of host Lauraceae by *X. glabratus*. Those cues also include attractive volatiles from the fungal symbiont (Hulcr et al. 2011), and a combination of fungal- and host-based odors has been shown to capture more *X. glabratus* than host odors alone (Kuhns et al. 2014b). Further research is needed to evaluate efficacy of multi-component lures (e.g., cubeb + eucalyptol + fungal odors).

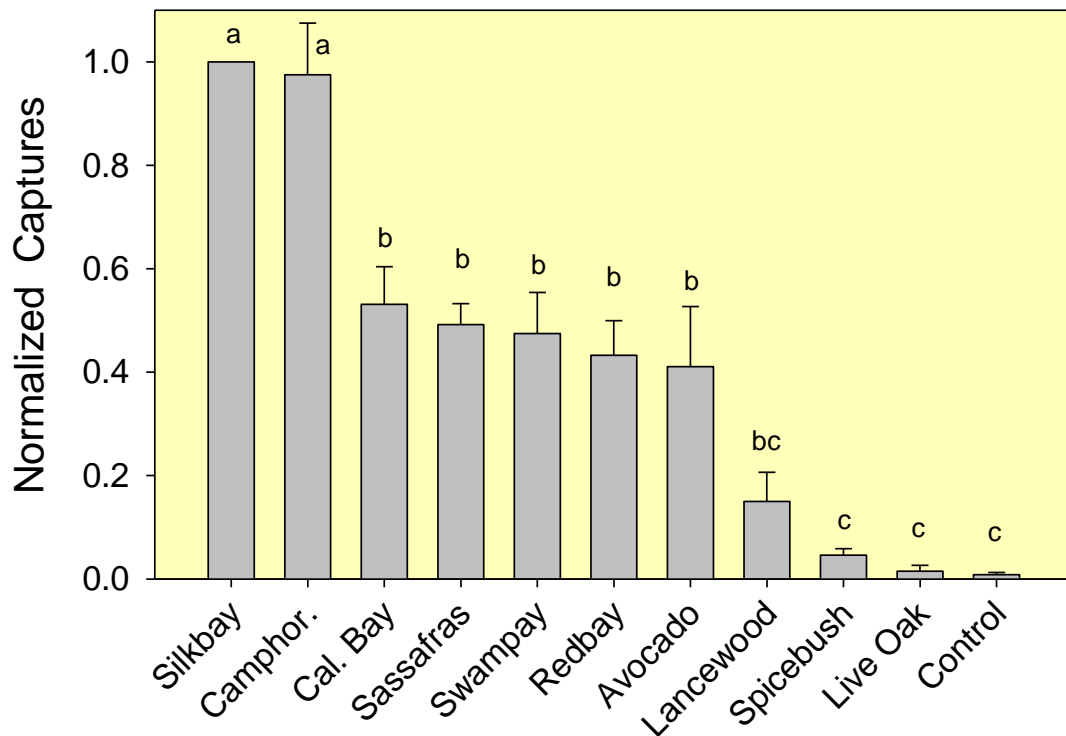


Figure 13. Relative attraction of *X. glabratus* to nine species of Lauraceae, as indicated by captures in sticky traps baited with wood bolts. Treatments also included live oak (*Quercus virginiana*, a non-host) and an un-baited control trap. Graph presents composite results from two replicated field tests; therefore, captures were normalized by converting to percentages relative to silk bay, the most attractive species, used as a positive control in each test. (Adapted from Kendra et al. 2014a).

## Diagnosis of the pathogen

For use in plant diagnostic laboratories, the National Plant Diagnostic Network (NPDN) developed a standard operating procedure for the diagnosis and identification of *R. lauricola* and *X. glabratus* (Harmon and Brown 2011, Harmon 2014 [available upon request]). This report documents the steps for proper packing and shipment of samples to diagnostic labs, descriptions of laurel wilt symptoms, *X. glabratus* identification, fungal isolation instructions, DNA extraction methods and PCR identification protocols. The first step in a laurel wilt diagnosis is the isolation of *R. lauricola* from host wood, which can be done by surface disinfestation of discolored wood chips by dipping into 95% ethanol and flaming or by dipping in a 10% bleach solution and placing on CSMA media (cycloheximide-streptomycin malt agar) (Harrington 1981, Fraedrich et al. 2008, Harmon 2014). Diagnostic morphological characteristics can be obtained from Harrington et al. (2008) and Harmon (2014). After isolation onto media, DNA extraction and PCR amplification is conducted for positive confirmation. Previous to 2014, molecular detection relied on the primers developed for the small subunit (SSU, 18S) of the ribosomal DNA (rDNA), the primers LWD1/LWD3 for standard PCR and LWD3/LWD4 for real-time PCR (qPCR) (Harmon and Brown 2011, Dreaden et al. 2014, Harmon 2014). However, in 2009, an undescribed *Raffaelea* species (PL 1004) was isolated from an avocado tree suspected to have laurel wilt. It was 99% similar in SSU sequence to *R. lauricola*, but not pathogenic (Dreaden et al. 2014, Harmon 2014, R. C. Ploetz, unpublished). The qPCR primers LWD3/LWD4, are specific to *R. lauricola* and *Raffaelea brunnea* T.C. Harr. (an ambrosial symbiont of the oak ambrosia beetle, *Monarthrum* spp.); however, late in the amplification process other *Raffaelea* species can also be amplified, but the method has some experimental utility (Dreaden et al. 2014). Thus, these primers were abandoned for diagnostic purposes.

To improve the detection of *R. lauricola* and limit the possibility of non-specific amplification of unknown species, a PCR assay was developed with two taxon-specific simple sequence repeat (SSR) loci. It is compatible with standard and qPCR methodologies and has a low detection limit (0.1 ng) when DNA is extracted from a fungal culture (Dreaden et al. 2014, Harmon 2014). This detection method is now being used by diagnostic labs at the University of Florida Plant Diagnostic Center and recommended within their standard operating procedure (Harmon 2014).

Since taxon-specific primers did not exist before Dreaden et al. (2014), positive confirmation of *R. lauricola* would often rely on the sequence analysis of the large subunit (LSU, 28S) and/or small subunit (SSU, 18S) of the rDNA. Unlike the majority of fungal species, amplification and sequencing of the Internal Transcribed Spacer (ITS) region (and sometimes the LSU) of the rDNA is problematic for *R. lauricola* due to the presence of high GC content and the formation of secondary structures (Fraedrich et al. 2008, Jeyaprakash et al. 2014). Jeyaprakash et al. (2014) established a Taqman qPCR probe based on the LSU rDNA region where PL1004 and *R. lauricola* have 100% sequence homology, implying the method will detect PL1004 and *R. lauricola* equally well, possibly resulting in false positives (T. J. Dreaden, personal communication). This method was found to successfully amplify the pathogen from cultures and/or infected wood samples and is currently in use for detection by the Florida Division of Plant Industry (along with confirmatory pathogenicity testing for avocado samples) (Jeyaprakash et al. 2014). The authors' also described a modified PCR protocol to allow for amplification and sequencing of a portion of the LSU and ITS regions (Jeyaprakash et al. 2014).

## **V. Response**

As a new and emerging threat to forests of the United States, the response to this disease is under the supervision of USDA-APHIS-Plant Protection and Quarantine agency (USDA-APHIS-PPQ),

delegated from the Secretary of Agriculture under the Plant Protection Act of 2000.

*Xyleborus glabratus* was first detected in USDA Forest Service Early Detection & Rapid Response (EDRR) Pilot Project traps in Port Wentworth Georgia in 2002. This trapping initiative was conducted by USDA Forest Service to monitor new and invasive species that may be entering the country through our ports, and ethanol lures were typically used to trap beetles. Initially, three female *X. glabratus* beetles were captured in proximity to warehouses near Port Wentworth. To assess the extent of the *X. glabratus* population within the area, additional traps were placed in coordination with APHIS. No other females were caught and thus beetles were suspected to have emerged directly from solid wood packing material (a common pathway for wood boring insect introductions) and not from local infested trees (Mayfield et al. 2009). Although ethanol lures are effective for trapping most ambrosia beetles, it is now known that these lures are not effective for trapping *X. glabratus* and initial populations may have been underestimated.

During 2003-2004 mortality of redbays trees was observed at Hilton Head Island, SC and neighboring localities in South Carolina and Georgia. Subsequent examinations revealed a common symptomology among wilted trees that included: rapid crown wilt, vascular discoloration, evidence of ambrosia beetle attack and eventually the consistent presence of *X. glabratus* and a new *Raffaelea* associated with the discolored xylem of affected trees. Researchers determined that the causal agent of the disease was a species of *Raffaelea*, later named *Raffaelea lauricola* (Harrington et al. 2008) and was transmitted by *X. glabratus*, and the new disease was named laurel wilt (Fraedrich et al. 2008).

Two symposia (2007 and 2009) were hosted by the Georgia Forestry Commission and the USDA Forest Service, Forest Health Protection with participants from the USDA Forest Service, universities, state agencies and other interested parties to exchange preliminary information on disease distribution, monitoring, biology and management. An *ad hoc* Laurel Wilt Working Group was formed in 2007 among

university, state, and federal scientists to facilitate gathering and dissemination of information on laurel wilt disease and discuss research needs, but is no longer active.

A laurel wilt website was established by the USDA Forest Service to inform researchers and the public about laurel wilt distribution, biology, management, contacts and advances in research. Links to research articles and other reports can be found at the laurel wilt website as well as a county wide distribution map, which thru the cooperation with state, university, and federal contacts tracks the spread of the disease (see XII. Web Resources). State forestry agencies (AL, FL, GA, MS, NC, SC) and universities have created webpages for laurel wilt to inform the public on the various aspects of the disease and its management, as well as information on what to do if laurel wilt is suspected in the landscape (see XII. Web Resources). A campaign for laurel wilt of avocado, "Save the Guac" (see XII. Web Resources) has also been launched to inform residents of dangers that this disease poses to the Florida avocado industry, along with a hotline to notify agents of possible laurel wilt sightings.

Additionally, extensive scientific research has been conducted by various federal, state and university scientists into the various facets of laurel wilt including: ecosystem impacts, host propagation and resistance, chemical management of the disease and vector, disease biology, epidemiology and expansions of host range, vector biology and chemical ecology, disease transmission, and many other aspects. The research resulted in preliminary management guidelines, which include the following (more detailed guidelines are included below):

Preventative fungicidal treatments of high value trees with propiconazole via macro infusion are currently the best method of protection; however, longevity is limited and application must be done professionally (Mayfield et al. 2008b). Management guidelines are provided within the appropriate state/federal agency and extension websites. In general, it is suggested that trees with laurel wilt be taken down and chipped to limit the reproduction of the vector (Spence et al., 2013) (tree stumps can support *X. glabratus* reproduction and should be removed or ground down). If chipping is not possible,

felled trees or logs should be left on site and covered with plastic or buried, if feasible. If laurel wilt is widespread within an area, logs and other infested woody material can be taken to a county dump where trees can be buried. Long distance transport of this material is discouraged as it aids in the expansion of disease to new areas (Crane and Smith 2010).

## **VI. Permits and Regulatory Issues**

As stated on the USDA-APHIS webpage: (see XII. Web Resources) “USDA regulates the importation and interstate movement of plant pathogens by requiring permits (codified at 7 CFR 330.200 to 330.212). Plant pathogens also include non-genetically engineered infectious substances, which can directly or indirectly injure, cause disease, or damage in any plants, plant parts, or plant products. If the organism is imported on/in host material, no separate permit is required if the host material is not intended for propagation”. [Dontmovefirewood.org](http://Dontmovefirewood.org) details specific state regulations for the transit of firewood (see XII. Web Resources). The encouragement for use of locally harvested and bought firewood is universal among host states, with a transport of less than 50 miles recommended (10 miles ideal). For the states currently affected by laurel wilt, other than the movement out of quarantined states into their respective states which is illegal, no specific regulations exist for AL, LA, MS and SC. Although regulatory actions have been taken to restrict the movement of wood that could spread other pests, no such actions have been taken to restrict the movement of *X. glabratus* and *R. lauricola* into new areas. Although these regulations do not apply to laurel wilt, any actions to lessen the movement of firewood will, by default aid in the suppression of laurel wilt spread. The Florida Department of Agriculture and Consumer Services, Division of Plant Industry enacted Rule 5B-65 (The Firewood and Unprocessed Wood Products legislation) in 2010 to suppress the movement of laurel wilt infested wood and wood products (see XII. Web Resources). In brief, movement of regulated articles requires permits and certificates of inspections (depending if classified as commercial or non-



commercial), firewood and unprocessed wood products are exempt if remaining within 50 miles of point of origin, and for the protection of the avocado production areas no firewood produced outside Miami-Dade County may enter unless certified.

## **VII. Cultural, Economic and Ecological Impacts**

### Cultural Impacts

The Lauraceae contains several plant species that play a central role to the medicinal and cultural activities of American Indian communities across North America (sassafras, California bay laurel, redbay and swamp bay). The Seminole and Miccosukee Tribes in Florida depend on redbay and swamp bay for more than 90% of traditional medicinal concoctions and the plants are used in a majority of significant cultural celebrations and ceremonies (Snow and Stans, 2001). Extinction of redbay and/or swamp bay would lead to the loss of its use by Seminole and Miccosukee communities, who have been dependent on this ingredient for generations. The tribes are concerned about this loss of cultural heritage (Chief Billie, 2014) and tribal foresters are looking into ways to mitigate the loss. Propagation of redbay and swamp bay trees from tribal lands is underway at the University of Florida and new studies are planned to evaluate the use of coppice regeneration to maintain plants for cultural use (J. Smith, personal communication).

### Economic Impacts

Redbay and other forest host species are generally not considered economically important; however, the avocado industry is in peril and is covered within its own USDA Recovery Plan (Ploetz et al. 2011a). However, due to the abundance of redbay and other native host trees within certain regions, their mortality and management potentially will have substantial economic impacts, including:

- The cost of tree removal and disposal for residential homes, state and city parks, roadways, municipalities, and other instances where trees cannot be left standing on-site,
- Loss of residential property value for homeowners who lost large, aesthetically pleasing trees, and/or the costs for replanting where it is mandated by statutes,
- Increase in administrative costs for state and local governments for materials and time associated with educating the public about laurel wilt and monitoring its spread,
- Possible loss of revenue for businesses that deal with firewood, mulches, and other unprocessed wood products due to increased regulation in movement and loss of viable wood supply due to the disease,
- Loss of revenue for the nursery industry that deals in the sale and propagation of these lauraceous species due to possible stop on sale orders from material deemed infected and from the loss of potential sales from homeowners and agencies that choose not to purchase these species for fear of laurel wilt,
- Fire hazards from increased fuel loads due to dead trees within natural areas, and
- Possible temporary restricted access due to hazardous decomposing trees killed by laurel wilt.

### Ecological Impacts

Laurel wilt has decimated members of the Lauraceae in the southeastern United States. Among the native laurels, none have been more affected than the aromatic native trees redbay and swamp bay. Laurel wilt causes rapid mortality of redbay and relatively few trees over 2.5 cm in dia survive by a few years after infestation (Fraedrich et al. 2008, Shields et al. 2011, Evans et al. 2013, Spiegel and Leege 2013). The sudden and substantial mortality of a once abundant tree has changed the species composition in affected areas, with redbay being replaced by other tree species (Goldberg and Heine 2009, Gramling 2010, Shields et al. 2011, Evans et al. 2013, Spiegel and Leege 2013). Behavioral studies

show that *X. glabratus* prefers to attack large diameter trees, but all size classes are susceptible to attack (Fraedrich et al 2008, Cameron et al. 2012, Maner et al. 2012 & 2014, Kendra et al. 2013a, Mayfield and Brownie 2013). Trapping studies have found that after the initial wave of laurel wilt mortality, *X. glabratus* populations decline dramatically as suitable host materials become scarce (Hanula et al. 2008, Cameron et al. 2012, Maner et al. 2014). However, in the absence of large host trees *X. glabratus* persists in smaller dia hosts (> 3.0 cm) and as stump sprouts mature they will likely be re-attacked to support newer generations (Cameron et al. 2012, Maner et al. 2014). Redbays re-sprout readily from the base and this mechanism allows for some persistence of the trees, with follow-up monitoring of these basal sprouts suggesting good rates of survivability thus far (R. S. Cameron, S. W. Fraedrich and J. Eickwort, personal observations). However, a study by Evans et al. (2013) tracked the fate of re-sprouts within St. Catherines Island (GA) and found 78% mortality among basal sprouts, likely due to laurel wilt and deer browsing, suggesting that these sprouts may not be a viable method to maintain redbay populations in unique island habitats with high deer populations.

Sassafras is also susceptible to laurel wilt and capable of supporting broods of *X. glabratus* (Riggins et al. 2011, Cameron et al. 2010). Although widely dispersed and small in size on the coastal plain, sassafras becomes a more important component of the forest in the southern piedmont and Appalachian Mountains, where they reach their greatest size (Griggs 1990, Koch and Smith 2008). Because of the clonal growth form of sassafras and apparent ability of *R. lauricola* to move in its lateral roots, it is likely that even low populations of *X. glabratus* will result in extensive mortality of this species. The ecological impacts of losses in this host are not yet known.

Redbay was a common hardwood of the coastal plain and maritime hammocks. It can grow to a height of 18 to 21 m and a diameter of 60 to 90 cm (Brendemuehl 1990). Redbay fruit serves as a food source for a variety of birds, rodents, bears and other mammals. Redbay leaves are used by many insects, including the specialized redbay psyllid (*Trioza magnoliae* Ashmead) (Hall 2012). The Palamedes

swallowtail (*Papilio palamedes* Drury) and the spicebush swallowtail (*Papilio troilus* L.) butterflies rely on lauraceous species as hosts (Lederhouse et al. 1992, Hall and Butler 2013a,b), and thus may also be negatively impacted by laurel wilt. The range of the Palamedes swallowtail butterfly mirrors that of redbay and concern over the declining redbay populations led researchers to test the potential for host shifting from redbay to camphortree (another species that supports larval development). Results indicated that *P. palamedes* does not discriminate between camphor trees and inanimate objects when choosing an egg-laying site, even though leaves fostered moderate levels of larval performance. This suggests that although *P. palamedes* could develop on camphor, the chances for a successful host shift are minimal since the butterflies do not preferentially choose this tree species as an egg-laying site (Chupp and Battaglia 2014). In the absence of mature redbay material, the *P. palamedes* can survive on seedling and stump sprouted material, which is abundant in much of the redbay range affected by laurel wilt in GA (Cameron et al. 2012).

As laurel wilt proceeds southward into the Florida Everglades, swamp bay populations have been severely affected (Rodgers et al. 2014). Aerial mapping of laurel wilt was conducted by the South Florida Water Management District (SFWMD) and the National Park Service (NPS) in 2011 and 2013. The 2011 aerial survey showed 4,925 ha coverage of laurel wilt, and by 2013 the coverage had increased to 133,740 ha (Fig. 14) (Rodgers et al. 2014). Concerns exist that invasive plants such as Brazilian pepper (*Schinus terebinthifolius* Raddi) and Old World Climbing Fern (*Lygodium microphyllum* [Cav.] R. Br.) may quickly colonize the ecological niches once held by swamp bay (Snyder 2014, J.A. Smith, personal observation). In addition the abrupt loss of tree island canopy can reduce peat accretion rates. (Cahoon et al. 2003)

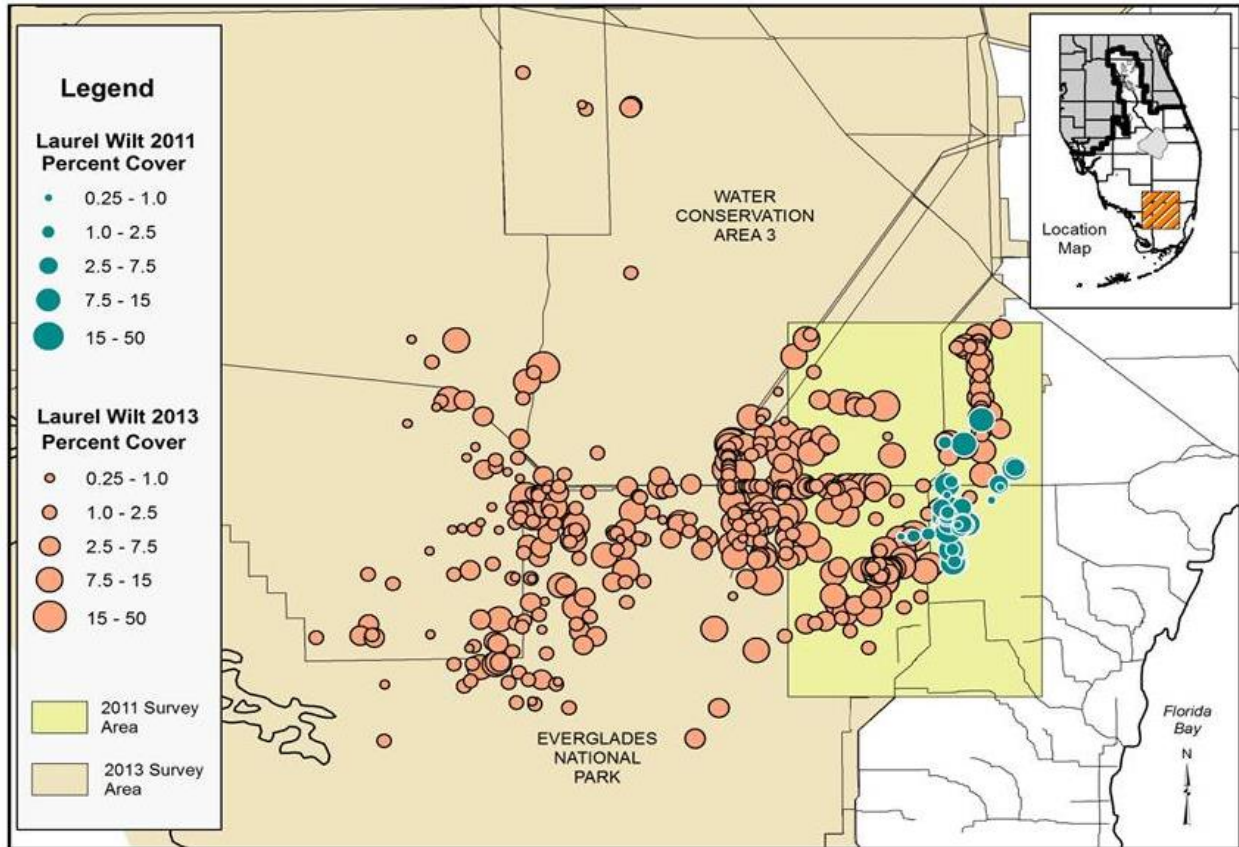


Figure 14. Florida Everglades laurel wilt distribution map for 2011 and 2013 (Adapted from Rodgers et al. 2014)

Surveys of wetland areas have reported mortality in the rare shrubs pondspice and pondberry due to laurel wilt, with *X. glabratus* using pondspice for brood material in FL (Surdick and Jenkins 2009, Fraedrich et al. 2011, Hughes et al. 2011). Additional monitoring in Florida has found no evidence of laurel wilt within the state's natural populations of bog spicebush (*Lindera subcoriacea* B.E. Wofford), northern spicebush (*Lindera benzoin*) and gulf licaria (*Licaria triandra*), although northern spicebush and Gulf licaria have been shown to be susceptible to *R. lauricola* via artificial inoculation (Fraedrich et al. 2008, Surdick and Jenkins 2010, Ploetz and Konkol 2013). Although susceptible to the pathogen, northern spicebush wood is not attractive to *X. glabratus* (Mayfield et al. 2013, Kendra et al. 2014a). Gulf licaria is represented as a single population in the United States (Simpson Park, Miami-Dade County

FL) where the establishment of laurel wilt within this tiny remnant population could result in extinction (Surdick and Jenkins 2010, Ploetz and Konkol 2013).

### VIII. Mitigation and Disease Management

Laurel wilt is now firmly established in the USA and its eradication not feasible. Management actions will focus on minimizing the effects of the disease within natural and residential areas, along with slowing its spread to new sites. Disease management options include the following:

#### Eradication/Sanitation

When laurel wilt was first detected on Jekyll Island (GA) in 2006, a concentrated effort was made to eradicate the disease from the island by the removal of and burning of symptomatic trees within the natural and residential areas of the island. Due to the ability of *X. glabratus* to colonize the remaining large asymptomatic redbays and freshly cut stumps (which were highly attractive to *X. glabratus*, J. Hanula, personal observation) on the island, laurel wilt returned by 2007 (Mayfield et al. 2009). Complete removal of laurel wilt and its vector may not be possible with sanitation methods alone, yet it could play a key role in the reduction of available brood material and *X. glabratus* population levels. Since large standing redbays can foster the development and emergence of *X. glabratus* for over a year, removal and destruction of these trees may help suppress the disease (Maner et al. 2013). In addition, due to the widespread distribution of the pathogen within infected trees, as well as attack and cohabitation within these trees by generalist ambrosia beetle species, dead standing redbays present an opportunity for other species to acquire *R. lauricola* via lateral transfer (Carrillo et al. 2014).

Spence et al. (2013) found that chipping infested logs reduced *X. glabratus* emergence by 99 percent. Chip piles covered with plastic tarps yielded no *X. glabratus* beetles, suggesting that this is an

effective method for preventing dispersal from infested host material. *Raffaelea lauricola* was successfully isolated from standing trees that had been dead for over a year (Spence et al. 2013) and *X. glabratus* can emerge from infested wood for over a year as well (Brar et al. 2013, Maner et al. 2013), while neither were recovered from wood after 2 days post chipping, suggesting that chipping greatly reduces pathogen and vector persistence in wood (Spence et al. 2013). Although reducing the likelihood of *X. glabratus* emergence, chipped material should not be transported to areas free of laurel wilt because some adults may survive the chipping process or may be attracted to or harbored in chip piles. Similar to previous recommendations for logs or fallen trees, chipped materials should be left on site (covered if possible) or taken to nearby municipal dumps where infested chips can be buried.

The removal of live or dead redbays in proximity to the rare and sub-optimal hosts pondspice and pondberry has been suggested as a way to reduce the number of *X. glabratus* in the area to lower the probability of attacks on these threatened and endangered species (Fraedrich et al. 2011). Strategic coppicing of host trees may also allow small, discontinuous populations to exist, while allowing the regrowth of uninfected sprouts after the disease has passed. However, these measures are probably not feasible for large land areas.

To be effective, attempts at sanitation by removal of dead and infected host material will require constant monitoring of disease progression within affected areas and adjacent uninfected areas, along with coordinated action in a timely manner. These methods have proven effective as part of an Integrated Pest Management program for Dutch elm disease, in which substantial differences in disease incidence existed between cities that did and did not implement some form of sanitation and disposal (Gibbs 2001).

## Protection

Various trapping studies have shown that *X. glabratus* generally flies within a few meters of ground level, with the majority of trap catches concentrated below 1.5 m on the trunk (Hanula et al. 2011, Brar et al. 2012). To evaluate the effectiveness of physically protecting the tree, screen barriers were used to exclude *X. glabratus* from accessing the lower trunk. Unfortunately, all screen-enclosed trees were killed by laurel wilt as beetles were able to bore through the barriers or simply attack the non-wrapped upper portions of the trunk (Maner et al. 2012).

Root graft transmission is suspected as a possible avenue of disease transmission in sassafras and avocado due to observed directional patterns of disease movement without evidence of beetle attack. As in other vascular wilt diseases (Dutch elm disease and oak wilt, caused by *Ophiostoma novo-ulmi* Brasier and *Ceratocystis fagacearum* [Bretz] Hunt, respectively) disruption of root grafts is an effective way to halt disease spread from tree to tree (Epstein 1978). Severing root connections generally involves the use of heavy mechanical equipment (trenchers and rock saws). Although not currently applied as a management technique for laurel wilt, this method may be useful for sassafras whose root system typically are interconnected through root sprouted trees.

### Chemical Control

Preventative control of laurel wilt on artificially inoculated redbays has been demonstrated by the use of the fungicide propiconazole via root flare injections (macroinfusion) (Mayfield et al. 2008b). If done correctly, the macroinfusion process results in a uniform distribution of the fungicide within the tree, thereby resulting in whole tree protection. This treatment has been adopted by commercial arborists for the protection of high value residential redbays (D. Spence, personal communication). Unfortunately, this protection has a limited duration of activity, with trees receiving the treatment often becoming vulnerable to laurel wilt within 1 to 1.5 years (A. E. Mayfield and D. Spence, personal communication). In theory, trees will require additional applications of the fungicide; however, little is



known about how many treatments will be needed (and tolerated by trees) for long term protection. Propiconazole was also used therapeutically, where a tree with laurel wilt ( $\leq 20\%$  canopy) in Gainesville FL was saved by the removal of the infected branch  $\approx 1$  m ahead of the vascular discoloration, use of a tree wound sealant, and reapplication of the fungicide, resulting in the halt of wilt (J. A. Smith, personal communication).

Ploetz et al. (2011b) investigated the effects of other fungicides and modes of application (soil drenches and bark sprays) on potted avocado trees. Drench applications of fungicides such as demethylation inhibitors (DMIs) and thiabendazole were found to reduce laurel wilt development, with the triazole class of fungicides showing the greatest potential. Topical branch sprays of propiconazole with Pentra-Bark™ (surfactant) and triadimenol were also protective to potted avocados (1.5 m tall) inoculated with *R. lauricola*; however, this is not effective on large diameter trees with thicker bark (R. C. Ploetz, unpublished)

Several synthetic insecticides have been tested for control of *X. glabratus* and other ambrosia beetle species in various assays with field grown swamp bays, potted avocados, and avocado bolts. Zeta-cypermethrin + bifenthrin and lambda -cyhalothrin + thiamethoxam provided the most consistent control of these wood boring beetles (Peña et al. 2011). A follow-up study was conducted to determine lethal concentrations (LC<sub>50</sub> & 90) of selected contact insecticides, along with their persistence in Florida's field conditions on avocado (Carrillo et al. 2013). In dipped bolt assays, a concentration-dependent relationship was observed in the mortality of *X. glabratus* with most insecticides tested. Complete mortality of *X. glabratus* was observed at the 2<sup>nd</sup> highest to highest test concentrations of: bifenthrin, permethrin, fenpropathrin, z-cypermethrin + bifenthrin, and I-cyhalothrin + thiamethoxam, chlorpyrifos and malathion. The percentage of *X. glabratus* found surviving in 15 d old excavated galleries was also concentration dependent for most insecticides. In field experiments, only malathion and z-cypermethrin + bifenthrin caused higher mortality than the control treatments after 15 d, but none of

the insecticides affected *X. glabratus* mortality or boring activity after 22 d (Carrillo et al. 2013). Results from these experiments indicate that although useful in *X. glabratus* suppression, the efficacies of these insecticidal treatments are short-lived. In addition, the threshold of unsuccessful protection of a live tree is a single beetle, and thus improvements in insecticidal control are needed before insecticides can be used a stand-alone option. The use of fungicides and insecticides has merit in the protection of high value trees; however, their use on a forest or landscape scale is unfeasible due to costs, re-application needs, unknown effects to non-target species, and risks to the environment.

#### Limit Movement of Infected Wood Material

Human assisted movement of *X. glabratus* infested wood was documented in Florida in 2008, when a citizen transported cut redbay logs from Duval to Volusia County for wood turning purposes, unknowingly establishing a new disease focus within the county (A. E. Mayfield, personal communication). The movement of unprocessed wood has also been suspected in the appearance of laurel wilt in areas far from the leading edge of the disease front like in AL, LA and MS. Progress has been made in educating the public on the environmental dangers of the movement of infested wood materials (see [Dontmovefirewood.org](http://Dontmovefirewood.org)). The reduction of human-aided transport can have significant impacts in eliminating large jumps in the distribution of laurel wilt. Possible mechanisms for reducing the human assisted spread of the disease include:

- Restrict the movement of redbay or other documented hosts out of infected counties,
- Increase public awareness about the possibilities of spreading laurel wilt through the movement of firewood and other unprocessed wood products,
- Assess the movement of unprocessed wood by the forestry industry to determine their possible role in disease spread, and
- Encourage rapid felling and chipping of trees killed by laurel wilt.

## Host Resistance

A research program for the evaluation of natural resistance to laurel wilt in redbay has been ongoing since 2007 at the University of Florida (Hughes 2013). In brief, researchers have established monitoring plots in areas of severe laurel wilt mortality in FL, GA and SC. Asymptomatic trees (> 3" DBH) on these sites have been vegetatively propagated (Hughes and Smith 2014) and tested in disease screening trials (Hughes 2013). A few redbay clones from SC and FL have demonstrated tolerance to laurel wilt in field trials, and additional trials and genotypes are underway (Hughes 2013). Operational testing for the assessment of horticultural traits from these superior clones is scheduled for early 2015 (J. A. Smith, personal communication). Next steps include the use of these clones in pilot restoration studies in areas currently experiencing high rates laurel wilt mortality to assess their performance under natural conditions (natural *X. glabratus* attack). Preliminary studies have begun to explore the possibility that surviving redbays may lack volatile signals that render them attractive to the vector (Hughes et al. unpublished).

Due to the environmental impacts upon the swamp bay within the Florida Everglades and upon tribal lands of the Seminole and Miccosukee Indians, germplasm preservation and resistance screening programs for this species are in development (J. A. Smith, personal communication). Sassafras may also be a candidate for disease resistance trials.

## Conservation of Germplasm

A redbay seed collection and storage initiative by the USDA Forest Service National Seed Lab was started in 2007 to gather and test various long term preservation methods (see XII. Web Resources). Due to high lipid content, redbay seed can only be stored for approximately 24 months (at °1-2 C) using traditional methods, with viability decreasing significantly with time (Vankus 2009). This scenario may

also be true for other laurel wilt susceptible species since seeds from members of the Lauraceae are generally temperature sensitive, and storage at sub-freezing temperatures usually results in seed mortality.

Vegetative propagation methods have been developed for swamp bay and redbay, making seed no longer the only focus of conservation efforts (Dehgan and Sheehan 1991, Hughes and Smith 2014). A germplasm collection of putatively resistant and susceptible redbay clones has been established at University of Florida locations in Gainesville and at the Plant Science Research and Education Unit in Citra, FL. In addition to serving as a repository for living redbays from ecotypes along the Atlantic Coastal Plain, inoculation trials have classified differing tolerance levels which can facilitate in depth studies on resistance and host-parasite-vector interactions among clones.

#### Integrated Pest Management

Slowing the spread of laurel wilt and protecting high value specimens is possible. The best approach will rely on multiple integrated methods. The following techniques may be applied in the management of laurel wilt:

- Monitoring and scouting for wilted or dead trees to assess the leading edge of the disease,
- Sanitation by the removal and chipping of dead logs or trees,
- Protection of high value trees by the use of fungicides and insecticides,
- Continued educational efforts on the impacts of the disease and how to prevent the spread of laurel wilt through the movement of infested wood, and
- Continued conservation of germplasm, development of resistant plant material, and alternative treatment methods.

## IX. Infrastructure and Experts

The following personnel have in depth knowledge and/or experience with the laurel wilt disease system on redbay and other forest hosts:

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## X. Research, Extension, and Education Needs

### Research Needs

- Discovery of new chemical control methods (fungicides and insecticides), including the extension of efficacy for propiconazole macroinfusion treatments. Assessment of fungicides for therapeutic uses,
- Develop *X. glabratus* attractants that are more attractive than the host trees,
- Investigate the role of root graft transmission for sassafras, avocado and other species sharing a connected root system,
- Delineate the susceptible host range of *R. lauricola* and *X. glabratus* within the Lauraceae around the world in order to forecast possible epidemics in other regions,
- Molecular comparisons between *R. lauricola* and other closely related species to help discover possible virulence factors,
- Compare chemical and structural differences between members of the Lauraceae that have shown susceptibility and resistance to laurel wilt,
- Investigate *X. glabratus* and *R. lauricola* interactions with hosts within its native range,
- Study the biology of *X. glabratus* in its native range and determine population regulating mechanisms there, including natural enemies,
- Determine why laurel wilt does not occur within the vector and pathogen's native range in native hosts,
- Investigate potential biological control agents against the vector and/or pathogen,
- Continue the development of resistant redbay germplasm and re-introduction techniques,
- Develop resistance screening programs for sassafras and other lauraceous hosts,



- Explore the disease dynamics within the Florida Everglades, in sassafras forests and in rare hosts,
- Continue the development of disease movement prediction models and assess potential movement out of the Atlantic Coastal Plain,
- Expand studies onto the host seeking behavior of the vector, while developing management techniques based on potential findings,
- Continue the development of improved lure systems for *X. glabratus* monitoring efforts,
- Assess the natural long range movement capabilities of *X. glabratus*,
- Determine the effects of laurel wilt upon rare plant communities (pondspice and pondberry) and possible conservation methods,
- Develop propagation methods for other host species,
- Determine the role of other ambrosia beetle species in the laurel wilt epidemic and assess if lateral transfer of the pathogen and transmission by other species is occurring under natural conditions,
- Continue research on the movement of unprocessed host wood and their roles in disease spread, along with possible management mechanisms,
- Pathway analysis of how the beetle and fungus arrived in the US and from where. How to prevent the long distance spread to areas such as the West Coast and Central/South America, and
- Determine impacts of laurel wilt epidemics on native flora, fauna, and ecological processes.

### **Extension and Education Needs**

Various online sources exist for information about laurel wilt (see XII. Web Resources). The dissemination of this knowledge will assist in creating a well-informed public. Researchers, extension

agents and producers of print and online publications are encouraged to submit materials to the USDA Forest Service-Forest Health Protection laurel wilt website. In addition, any movement of the disease into new and undocumented counties should be reported to local state forest health coordinators so that confirmed new counties can be included on an updated distribution map on the website. State, government, and university scientists and forest health personnel are encouraged to participate in meetings and workshops to educate other researchers, agents, or concerned groups and individuals about laurel wilt and management options.

Efforts should be continued in educating the public and forestry industry about the potential dangers of the movement of firewood and unprocessed wood products and suggest best practice methods. Special attention should be given to campgrounds and campground owners in regards to firewood movement, with informational material and alternative methods of procuring safe wood freely available. Campers and residential homeowners should also be informed on the basic identification of laurel wilt, its symptoms and management strategies. Much of this information has been provided in the web resources section.

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## XII. Web Resources

-Alabama Forestry Commission - laurel wilt web page  
<http://www.forestry.alabama.gov/LaurelWilt.aspx?bv=3>

-Don't Move Firewood - laurel wilt web page  
<http://www.dontmovefirewood.org/gallery-of-pests/laurel-wilt.html>

-Florida Department of Agriculture and Consumer Services (FDACS) - laurel wilt web page  
<http://www.freshfromflorida.com/Divisions-Offices/Plant-Industry/Pests-Diseases/Laurel-Wilt-Disease>

-Florida Department of Agriculture and Consumer Services (FDACS) - Rule 5B-65, Firewood and Unprocessed Wood Products  
[www.freshfromflorida.com/content/download/24082/487223/pdf\\_firewood\\_rule\\_backgrounder.pdf](http://www.freshfromflorida.com/content/download/24082/487223/pdf_firewood_rule_backgrounder.pdf)

-Forest Health Protection, Southern Region, USDA Forest Service - laurel wilt web page and distribution map  
<http://www.fs.fed.us/r8/foresthealth/laurelwilt/index.shtml>

-Forest Health Protection, Southern Region, USDA Forest Service - laurel wilt and seed collection web page  
[http://www.fs.fed.us/r8/foresthealth/laurelwilt/seed\\_collection/seed\\_collection.shtml](http://www.fs.fed.us/r8/foresthealth/laurelwilt/seed_collection/seed_collection.shtml)

-Georgia Forestry Commission - laurel wilt web page  
<http://www.gfc.state.ga.us/forest-management/forest-health/laurel-wilt-disease/index.cfm>

-Louisiana Department of Agriculture and Forestry web page  
<http://www.ldaf.state.la.us/>

-Mississippi Forestry Commission - laurel wilt web page  
<http://www.mfc.ms.gov/laurel-wilt.php>

-North Carolina Forest Service - laurel wilt web page  
[http://ncforestservice.gov/forest\\_health/forest\\_health\\_laurelwiltfaq.htm](http://ncforestservice.gov/forest_health/forest_health_laurelwiltfaq.htm)

-Save the Guac, Florida Department of Agriculture and Consumer Services (FDACS)  
[www.savetheguac.com](http://www.savetheguac.com)

-South Carolina Forestry Commission - laurel wilt information pdf  
<http://www.state.sc.us/forest/idwilt.pdf>

-Southern Plant Diagnostic Network  
<http://www.sepdn.org/>

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) – plant pathogens web page  
[http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth/sa\\_import/sa\\_permits/sa\\_plant\\_pests/sa\\_plant\\_pathogens/ct\\_plant\\_pathogens!/ut/p/a0/04\\_Sj9CPykssy0xPLMnMz0vMAfGjzOK9\\_D2MDJ0MjDzd3V2dDDz93HwCzL29jAx8TfULsh0VAY\\_1WkE!/](http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/planthealth/sa_import/sa_permits/sa_plant_pests/sa_plant_pathogens/ct_plant_pathogens!/ut/p/a0/04_Sj9CPykssy0xPLMnMz0vMAfGjzOK9_D2MDJ0MjDzd3V2dDDz93HwCzL29jAx8TfULsh0VAY_1WkE!/)