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Xyleborus glabratus attacks and systemic colonization by *Raffaelea lauricola* associated with dieback of *Cinnamomum camphora* in the southeastern United States

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Summary

Laurel wilt, caused by *Raffaelea lauricola*, is responsible for extensive mortality of redbay and other American members of the Lauraceae in the southeastern United States. *Raffaelea lauricola* is a mycangial symbiont of the redbay ambrosia beetle (*Xyleborus glabratus*), and the beetle and fungus were accidentally introduced from Asia. Branch dieback of camphortree (*Cinnamomum camphora*), an Asian member of the Lauraceae, has been occasionally observed in areas where laurel wilt has decimated redbay populations, and *R. lauricola* was isolated from such camphortrees. However, the role of *X. glabratus* and *R. lauricola* in this branch dieback remains unclear. Examination of camphortrees on Jekyll Island, Georgia showed that healthy-appearing trees and those with branch dieback had been attacked by *X. glabratus*, but the trees with branch dieback had four times as many beetle attacks. *Raffaelea lauricola* was routinely isolated from discoloured xylem near beetle tunnels in healthy trees and those with dieback. Single-point inoculations with *R. lauricola* on stems of mature, healthy camphortree trees failed to induce wilt-like symptoms or branch dieback, although areas of discoloration were scattered throughout the xylem, and *R. lauricola* was reisolated irregularly at various heights in some inoculated trees. In growth chamber experiments, single-point inoculations with *R. lauricola* resulted in systemic colonization but no wilt symptoms or branch dieback in camphortree saplings. In contrast, inoculations at multiple points along the stem (simulating multiple attacks by the vector) caused branch dieback and wilt-like symptoms, including a brownish, diffuse discoloration of the xylem. Camphortree appears to be more resistant than American species of Lauraceae to the vascular wilt caused by *R. lauricola*. The fungus does colonize camphortrees systemically, however, and can apparently cause branch dieback. This suggests that the fungus may provide brood material for *X. glabratus* in Asia as it does in the southeastern United States.

1 Introduction

Laurel wilt, caused by *Raffaelea lauricola* TC Harrin., Fraedrich, & Aghayeva, is responsible for extensive mortality of redbay (*Persea borbonia)*, swampbay (*Persea palustris*, considered synonymous with *P. borbonia* by some) and sassafras (*Sassafras albidum*) in forests of the southeastern United States (Fraedrich et al. 2008; Harrington et al. 2008). The pathogen is spread by an ambrosia beetle, *Xyleborus glabratus*, which is believed to have been introduced into the United States near Savannah, Georgia, in the late 1990s or early 2000s (Hanula et al. 2011). Since that time, *X. glabratus* and *R. lauricola* have spread rapidly in maritime and coastal plain forests, which often have redbay as a major component. Laurel wilt is now present in much of Florida and is found as far west as Mississippi and as far north as North Carolina. In addition to redbay and sassafras, other Lauraceae indigenous to the southeastern United States are also highly susceptible to the wilt, including spicebush (*Lindera benzoin*) (Fraedrich et al. 2008), pondberry (*Lindera melissifolia*), pondspice (*Litsea aestivalis*) (Fraedrich et al. 2011) and other *Persea* spp. (Fraedrich et al. 2008; Hughes et al. 2012). The effect of *R. lauricola* infection is more variable on avocado (*Persea americana*) (Fraedrich et al. 2008), a species that is indigenous to Mexico and Central America but grown commercially in Florida. Some cultivated avocado varieties appear to be somewhat resistant to the wilt, while others are more susceptible (Ploetz et al. 2012).

Xyleborus glabratus is native to Asia, where it has been found in Japan, Taiwan, India, Myanmar and China (Wood and Bright 1992; Hulcr and Lou 2013). In Asia, the beetle is associated with aromatic tree species, especially members of the Lauraceae such as *Cinnamomum camphora* (Hulcr and Lou 2013), *C. osmophloem* (Harrington et al. 2011), *Lindera latifolia* and *Litsea elongata* (Wood and Bright 1992). *Raffaelea lauricola* is a fungal symbiont of *X. glabratus* and is the predominant species in the mycangia of this beetle in the USA, Taiwan and Japan (Fraedrich et al. 2008; Harrington and Fraedrich 2010; Harrington et al. 2010, 2011). However, there are no reports that indicate *R. lauricola* causes a plant disease in Asia, and Asian Lauraceae may be highly resistant to the disease.

Most species of ambrosia beetles live in the xylem of recently dead or highly stressed woody plants (Wood 1982), but in this regard, *X. glabratus* appears to interact differently with the American Lauraceae. In contrast to most ambrosia beetles, adult *X. glabratus* females bore into the sapwood of healthy plants and inoculate them with conidia of *R. lauricola*, which ooze from the mycangia during tunnelling. Initial attacks on plants by *X. glabratus* are aborted, but the fungus moves rapidly and systemically in the xylem of susceptible plants (Fraedrich et al. 2008). This results in the plugging of water-conducting tissues and wilt symptoms, presumably by the fungal tissues as well as by the production of tyloses and gums by the host (Inch and Ploetz 2012; Inch et al. 2012). Later generations of beetles make brood galleries in the wilted trees (Harrington and Fraedrich 2010).

Camphortree (*C. camphora*) is indigenous to Japan, Taiwan, Korea and China, as well as other countries in eastern Asia, and has been used for camphor production and also as an ornamental tree in warmer areas of the southeastern United States and in California. In some areas of the United States, camphortree has naturalized as a forest tree species (Langeland

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et al. 2008). Branch dieback and mortality have been observed in a few camphortrees in Florida and Georgia, and *R. lauricola* was isolated from such trees, suggesting that they were affected by laurel wilt (Smith et al. 2009). However, the susceptibility of camphortree to laurel wilt or dieback caused by *R. lauricola* has not been evaluated in controlled inoculations. Furthermore, widespread wilt and mortality in camphortree have not been observed. Most camphortrees appear healthy and branch dieback is relatively rare in coastal areas of Georgia, even at locations such as Jekyll Island, where extensive mortality due to laurel wilt has been observed in redbay and where high populations of *X. glabratus* have occurred (Hanula et al. 2008, 2011).

Thus, it is not clear if camphortree is susceptible to laurel wilt or if it can support *X. glabratus* brood development. We hypothesized that Asian Lauraceae in the native range of *X. glabratus* are susceptible to systemic colonization by *R. lauricola* and branch dieback, which would provide brood material for the beetle. However, Asian Lauraceae may be relatively resistant and not generally killed by the disease, and even branch dieback may only occur after multiple inoculations of *R. lauricola* due to numerous aborted attacks by *X. glabratus*.

The objectives of this study were (i) to evaluate the susceptibility of camphortree to laurel wilt and dieback following inoculation with *R. lauricola*; (ii) to determine whether *R. lauricola* moves systemically in the vascular system of camphortree saplings and trees; and (iii) to determine whether *R. lauricola* and *X. glabratus* are associated with healthy-appearing camphortrees and camphortrees exhibiting branch dieback in an area where laurel wilt has caused extensive mortality of redbay.

2 Materials and methods

2.1 Growth chamber inoculation studies

Camphortree saplings were inoculated with *R. lauricola* in a growth chamber study in July 2008 to determine their susceptibility to laurel wilt. Redbay saplings were also inoculated and served to verify the aggressiveness of the isolates. The saplings were approximately 3 years of age and were grown in containers with a commercial nursery potting mix. The mean height of camphortree saplings was 131 cm, and the mean diameter at groundline was 18 mm. The mean height of the redbay saplings was 138 cm, and the mean diameter at groundline was 13 mm. Two isolates of *R. lauricola* were grown on malt extract agar (MEA, 2.5% malt extract and 2.0% agar) at 25°C for 16 days prior to inoculations. One isolate (C2428, in the collection of T. C. Harrington) was obtained from a wilted redbay on Hilton Head Island, South Carolina, and the other was isolated from a recently wilted redbay in Brantley County, Georgia. Conidia were collected by flooding plates with approximately 20 ml of sterile deionized water, loosening conidia with a sterile glass rod and passing the conidial suspension through 3-ply sterile gauze. The gauze was then rinsed with another 20 ml of sterile deionized water. The concentrations of conidia as determined with a haemocytometer were 1.4×10^6 ml^{-1} for isolate C2428 and 2.3×10^6 ml^{-1} for the second isolate.

Fifteen camphortree and 15 redbay saplings were wounded by drilling 2-mm-diameter holes into stems at 4–7 cm above the groundline to a depth of approximately one-half the diameter of the stem. For each species, five saplings were inoculated with 0.2 ml of the conidial suspension from isolate C2428, another five saplings were inoculated with the second isolate, and 0.2 ml of sterile deionized water was placed into the wounds of the remaining five saplings. Inoculation points were wrapped with Parafilm M© (Pechiney Plastic Packaging, Menasha, WI, USA), and saplings were placed in a growth chamber at 26°C and 15 h of light per day. Plants were watered every 2–4 days, as needed. After 7 weeks, saplings were evaluated for wilt and xylem discoloration. Sections of stems from locations 10–20 cm above the inoculation points were surface disinfected by dipping briefly in 95% ethanol and flaming, and pieces of the stem tissues were plated on cycloheximide-streptomycin malt agar (CSMA, 1% malt extract, 1.5% agar, and 200 ppm cycloheximide and 100 ppm streptomycin sulphate added after autoclaving), a medium selective for *Ophiostoma* spp. and related anamorphs, such as *Raffaelea* spp. (Harrington 1981, 1992).

The experiment was repeated in June 2010. The mean height of the camphortree saplings was 81 cm, and the mean diameter at groundline was 15 mm. The mean height of redbay saplings was 153 cm, and the diameter at groundline was 26 mm. Two isolates of *R. lauricola* were used in this experiment; C2428 as previously described, and C2258 from a wilted redbay tree in the Timucan Preserve near Jacksonville, Florida. The concentration of conidia for each isolate was approximately 1.25×10^6 ml^{-1}. Fifteen camphortree and eight redbay saplings were wounded, and sets of five camphortree and three redbay saplings were each inoculated with the conidial suspension of one of the two isolates as previously described. Another set of five camphortree and two redbay saplings were mock-inoculated with sterile deionized water. Inoculation points on all saplings were wrapped with Parafilm, placed in a growth chamber with a 16-h photoperiod with daytime and night-time temperatures at 26°C and 24°C, respectively, and watered every 2–4 days, as needed. After 8 weeks, saplings were evaluated for disease symptoms and the presence of *R. lauricola* in stems.

A third experiment was conducted in August 2009 to evaluate the systemic movement of *R. lauricola* in camphortree, avocado and redbay during a 5-day period following inoculation. Ten saplings of each species were used. Their respective average height and diameter at groundline were 140 cm and 25 mm for redbay, 104 cm and 19 mm for camphortree, and 130 cm and 26 mm for avocado. One isolate (C2627) was from a *X. glabratus* beetle that had been extracted from a camphortree on Jekyll Island (concentration of conidia was 1.5×10^6 ml^{-1}), and the other isolate was from a symptomatic redbay in Chandler Co., Georgia (concentration of conidia was 6.7×10^5 ml^{-1}). All saplings were wounded at a point 7 cm above groundline with a 2-mm-diameter drill bit, and 0.2 ml of the conidial suspension from one of the two isolates was inoculated into five saplings of each species, and the remaining saplings of each species were inoculated with the other isolate. The wound sites were wrapped with Parafilm after inoculation, and the saplings placed in a growth chamber at 25°C

with a 16-h photoperiod. On each of 5 days after inoculation, two saplings of each species, one for each of the isolates of *R. lauricola*, were removed from the growth chamber, and 5-cm-long segments were cut from the stem, beginning at 7 cm above ground level and continuing at 18-cm intervals (i.e. 7–12, 25–30, 43–48, 61–66 cm, etc.) to the tops of the saplings. The sections of stems were surface disinfected as previously described, and pieces of stem tissues from each section were plated on CSMA plates, incubated at 25°C and evaluated after 7–10 days for growth of *R. lauricola*.

A fourth experiment was initiated in April 2013 to determine the effect of multiple-point inoculations along the stem of camphortrees. The saplings had an average height of 166 cm and a diameter of 25 mm at groundline. Two isolates of *R. lauricola* were used in the experiment, C2428, as previously described, and C2798 isolated from a wilted sassafras tree in Marengo Co., Alabama. The isolates were grown on MEA for 14 days, and conidia were collected as previously described. Concentrations of conidia for isolates C2798 and C2428 were 1.7×10^6 ml^{-1} and 2.0×10^6 ml^{-1}, respectively. Eighteen saplings were wounded by drilling 2-mm-diameter holes to a depth of approximately 1 cm into the stems. In nine of the saplings, five holes were drilled, spaced 7.5 cm apart up the stem, beginning at 7.5 cm above groundline. Each drill hole was placed at a 90 degree angle to the previous drill hole in a spiral pattern. The other nine saplings received ten drill holes, placed along the stem at 7.5-cm intervals. For each wound treatment and isolate, each drill hole on three saplings was inoculated with 0.1 ml of inoculum. Drill holes on all controls each received 0.1 ml of sterile deionized water. For each of the two isolates, two redbay saplings were inoculated into a single drill hole located 7.5 cm above groundline with 0.1 ml of inoculum to verify that the isolates were aggressive. All wound sites were wrapped with Parafilm. The saplings were placed in a growth chamber with a 15-h photoperiod, and the day/night temperatures were set at 26/24°C, respectively. Saplings were watered every 2–4 days, as needed, and were evaluated every 10–14 days for symptom development. After 8 weeks, a final evaluation was conducted to assess symptoms of wilt and xylem discoloration, and the presence of *R. lauricola* in stems was determined by isolation.

2.2 Susceptibility of camphortree trees – field study

Larger, naturalized camphortrees were inoculated in a forest near Waycross, Georgia (N31°12.068′; W082°21.589′). The 15 trees selected were 20–30 years old with heights ranging from 8.2 to 14.3 m and diameters at breast height (DBH, 1.3 m) ranging from 5.0 to 12.5 cm. All trees appeared healthy at the time of inoculation, and redbay trees were not observed in the stand of camphortrees or in the immediate vicinity. Two isolates of *R. lauricola* were used for inoculations, C2428 and C2792, which was obtained from a wilted sassafras tree in Jackson County, Mississippi. The isolates were grown on MEA for 2 weeks prior to inoculations. On 6 April 2011, the trees were randomly assigned to one of three treatments: five camphortrees were inoculated with isolate C2428, five trees with isolate C2792 and five trees were controls. Each tree was wounded by drilling a 6-mm-diameter hole 2–3 cm deep into the xylem at 1.3 m above the ground. A MEA plug (5 mm diameter) with mycelium and conidia of an isolate of *R. lauricola* or a sterile agar plug was placed into the wound of each tree. The inoculation site on each tree was wrapped with Parafilm, and then, duct tape was used around the stem to secure the Parafilm wrap. The trees were first examined for wilt and branch dieback on 23 June 2011, and two trees inoculated with *R. lauricola* and one control tree were cut down to examine for xylem discoloration. Discs (3–6 cm thick) were cut from the tree stems at heights of 3 m and 9 m, and the samples were placed in a cooler and transported to the Forest Sciences Laboratory in Athens, Georgia. In the laboratory, pieces of the xylem were plated on CSMA to determine the presence of *R. lauricola*. The trees were re-evaluated on 8 February 2012, and two trees inoculated with *R. lauricola* and one control tree were cut down and examined more intensively for xylem discoloration and the presence of *R. lau*ricola. Discs (3–6 cm thick) were cut from the stem at the groundline, at the inoculation point and at subsequent intervals of 1.5 m along the main stem to the tree tops (i.e. 3.0, 4.5, 6.0 m, etc. above groundline). The samples were placed in plastic bags and then in a cooler for transport to the laboratory. The discs were examined for xylem discoloration, and samples were plated on CSMA to determine the presence of *R. lauricola*. The stand was visited again on 8 June 2012, and the remaining six inoculated trees and three control trees were felled. Discs were cut from the main stems and examined for xylem discoloration and evaluated for the presence of *R. lauricola* by isolation on CSMA.

The aggressiveness of six *R. lauricola* isolates recovered from the camphortrees after 10 and 14 months was re-evaluated by inoculating redbay saplings. Three isolates from camphortrees inoculated with C2428 and three isolates from trees inoculated with C2792 were grown on MEA for 24 days. Conidia were collected as previously described and concentrations ranged from 2.6×10^6 to 7.6×10^6 ml^{-1} among isolates. The redbay saplings were 2 years of age and grown in containers with a commercial nursery potting mix. The mean plant height was 85.5 cm, and the mean diameter at groundline was 8.2 mm. The 21 saplings were divided into seven sets of three saplings each, and all plants were wounded by drilling holes (2.25 mm diameter, 4 mm deep) into the stem at 3–6 cm above groundline and inoculated with 0.1 ml of a conidial suspension or 0.1 ml of sterile water. Wounds were wrapped with Parafilm, and the saplings were placed in a growth chamber with a 16-h photoperiod, and day and night temperatures set at 26°C and 24°C, respectively. Plants were evaluated for symptoms at 10 days and again at 28 days after inoculation. After 28 days, pieces of sapwood were obtained from stems at 10–15 cm above the inoculation points, surface disinfected as previously described, and pieces of stem tissue were plated on CSMA and evaluated for the presence of *R. lauricola.*

2.3 Association of *Xyleborus glabratus* and *Raffaelea lauricola* with symptomatic and asymptomatic camphortrees

The incidence of *X. glabratus* and *R. lauricola* in the stems of camphortrees was evaluated at a site on Jekyll Island where naturalized camphortrees were a major component of the forest and where laurel wilt had been present for at least

4 years. The camphortrees were subdominant and midstory in the stand, and live oak (*Quercus virginiana*) and loblolly pine (*Pinus taeda*) were the dominant tree species. Bayberry (*Morella cerifera*) and magnolia (*Magnolia grandiflora*) occurred primarily as understory species. Five camphortrees with branch dieback and five other camphortrees that appeared to be healthy were cut down and examined between October 2010 and April 2012. The height, DBH and the general health (e.g. evidence of branch dieback of each tree, presence of wilted leaves, etc.) were recorded. Branches and small shoots were examined in the field for damage, and samples were collected from branches with recent dieback or where wilted foliage was observed. The main stem of the trees was cut into bolts approximately 75 cm in length, placed in plastic bags and transported in portable coolers. In the laboratory, the bolts were cut into discs 6.5 cm thick, and the cross section of each piece was examined for the presence of xylem discoloration. When discoloration was observed, the disc was further dissected with hammer, chisel and a knife to determine the cause of the discoloration (e.g. insect tunnel and branch stub). The number of beetle entrance holes that had diameters similar to those produced by *X. glabratus* was determined, and the number of holes on healthy-appearing camphortrees and those with branch dieback was compared with a standard t-test (Steel and Torrie 1980). The presence of beetles in tunnels was determined by forcing beetles from tunnels with a piece of straw, or sections of tunnels were split open using hand-pruning shears to expose the insides of tunnels.

Isolations of *R. lauricola* were attempted from sapwood samples with discoloration around beetle tunnels. The samples were surface disinfected by dipping them into 95% ethyl alcohol and flaming, and then placing small chips on CSMA. Samples for plating were similarly taken from areas away from tunnels where light staining or discoloration in the xylem could not be attributed directly to beetle tunnelling or other causes. Plates were evaluated for *R. lauricola* after 10–14 days.

Eight isolates obtained from areas of discoloration in the xylem of five camphortrees and identified as *R. lauricola* based on morphological characteristics were subsequently evaluated by PCR amplification and sequencing of the large subunit (LSU) rDNA, which is used to identify *Raffaelea* spp. (Harrington et al. 2010). Eight isolates were also tested for aggressiveness on redbay saplings. The isolates were grown on MEA for 21 days, conidia were collected and enumerated as previously described, and the concentrations of conidia ranged from 7.6×10^5 to 3.9×10^6 ml^{-1} among isolates. The container-grown saplings were 3–4 years old and had a mean height of 182 cm and a mean diameter at groundline of 20 mm. The 27 saplings (three replicates per isolate or control) used in the test were wounded by drilling holes (3.25 mm diameter, 5–10 mm deep) into the stem at 4–7 cm above groundline, and each sapling was inoculated with 0.1 ml of conidial suspension from one of each of the eight fungal isolates or with sterile deionized water. Inoculation points were wrapped with Parafilm, saplings were placed in a growth chamber with a 15-h photoperiod, and day and night temperatures were set at 26°C and 24°C, respectively. Wilt symptoms were evaluated after 16 and 27 days. On the latter date, plant stems were evaluated for sapwood discoloration. Pieces of stem tissue were obtained at locations 10–20 cm above the inoculation points, and wood chips were surface disinfected and plated on CSMA. Plates were incubated at 25°C and evaluated after 7–10 days for the presence of *R. lauricola*.

3 Results

3.1 Growth chamber inoculation studies

Camphortree saplings inoculated with *R. lauricola* did not exhibit xylem discoloration at the end of the first experiment, but six of the 10 plants had leaf loss and exhibited chlorosis in leaves on some shoots. Likewise, control plants did not exhibit xylem discoloration but also exhibited leaf loss and leaf chlorosis in some shoots of all plants. Thrips were common on the underside of the leaves and along twigs of inoculated and control plants, and specimens were identified by Dr. G. B. Edwards (Florida Department of Agriculture and Consumer Services, Gainesville, Florida) as *Liothrips floridensis*, an insect known to damage camphortree. Although no symptoms of laurel wilt were seen, *R. lauricola* was isolated from the xylem of three of the ten inoculated camphortree saplings at the end of the experiment. The fungus was not isolated from any of the control saplings. All redbay saplings inoculated with *R. lauricola* wilted (i.e. limp, drooping foliage, becoming brown and dying) and had xylem discoloration typical of laurel wilt. *Raffaelea lauricola* was isolated from all inoculated redbay saplings at the end of the experiment. Redbay saplings in the control treatment did not wilt and did not exhibit xylem discoloration, and *R. lauricola* was not isolated from the xylem at the end of the experiment.

At the end of the second experiment, neither xylem discoloration nor wilt was observed in the *R. lauricola*-inoculated camphortree saplings or the control saplings, although leaf loss and chlorosis were again observed in all inoculated and control plants. Thrips were again associated with the symptoms. *Raffaelea lauricola* was isolated from four of 10 inoculated camphortree saplings at the end of the experiment but was not isolated from any of the control saplings. All redbay saplings inoculated with *R. lauricola* had xylem discoloration and exhibited wilt, and the pathogen was isolated from all saplings. The redbay control saplings remained healthy, had no xylem discoloration, and *R. lauricola* was not isolated from the plants.

In the third experiment, *R. lauricola* moved rapidly upward in the xylem of redbay and avocado saplings, but the pathogen moved at a slower rate in camphortree saplings (Fig. 1). By day five, the fungus could be recovered throughout the stems of redbay and avocado at distances as great as 130 cm above the inoculation point, but in camphortree, *R. lauricola* could be only isolated at distances up to 60 cm from the inoculation point, and not all the plated chips yielded the fungus.

In the fourth growth chamber experiment, several camphortrees inoculated at multiple points with *R. lauricola* exhibited wilt-like foliage symptoms and shoot dieback as early as 10 days after inoculation. Some or all of the shoots of all camphortree saplings inoculated at five points with isolate C2428 showed wilt symptoms at 14 days, but only one of the three saplings inoculated with C2798 exhibited wilt symptoms. All camphortrees inoculated at 10 points along the stem exhibited

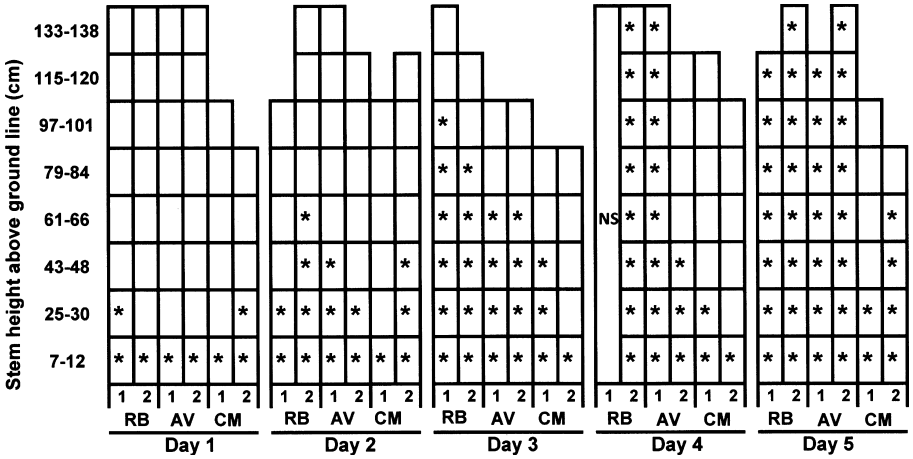

Fig. 1. Isolation of *Raffaelea lauricola* (asterisk) from inoculated saplings of *Persea borbonia* (RB; redbay), *Persea americana* (AV, avocado) and *Cinnamomum camphora* (CM; camphortree) at various stem heights at 1–5 days after inoculation at 7 cm above groundline. Areas not outlined as rectangles were above the height of the saplings at the time of sampling. Two saplings (numbered 1 and 2) of each species were sampled on each day except for one of the redbay saplings sampled on day 4 (NS), which was discarded because it was found to have a stem canker and xylem discoloration at 28 cm above groundline.

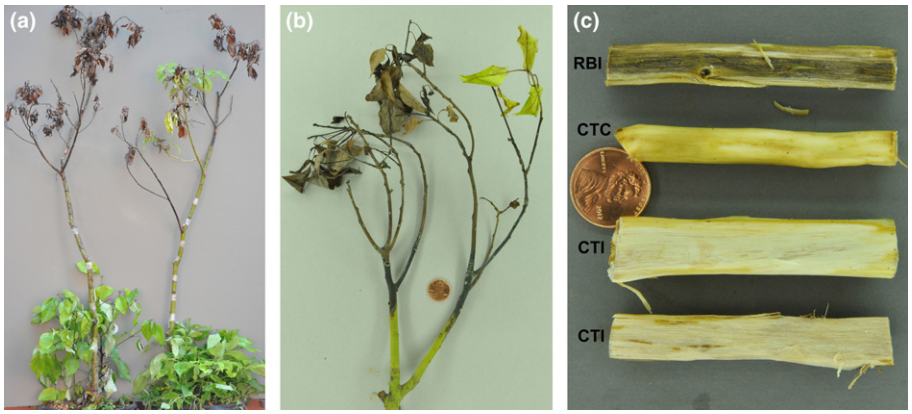

Fig. 2. Effects of multiple-point inoculations with *Raffaelea lauricola* on stems of camphortree. (a) Wilted saplings at 56 days after 5-point (left sapling) and 10-point (right sapling) inoculation; (b) wilted foliage and dieback-like symptoms with transitional area having dark black outer bark and dead phloem above, and healthy-appearing bark with green colour below; (c) dark black discoloration in xylem of redbay inoculated with *R. lauricola* (RBI), light to dark brown discoloration in xylem of inoculated camphortree saplings (CTI) and stem section of non-inoculated, healthy camphortree sapling (CTC).

symptoms at day 14. When plants were reassessed at 23 days after inoculation, all inoculated camphortree saplings exhibited complete or partial wilting, and discoloration was evident in the xylem of the symptomatic saplings. Most camphortree saplings had completely wilted (Fig. 2a) at 56 days, but the three saplings inoculated five times with isolate C2798 exhibited only partial crown wilt. Where branches appeared to have died, there was often an abrupt transition in the phloem and outer bark, with a dark black discoloration and necrosis above the transition zone and healthy-appearing phloem and bark below (Fig. 2b). The xylem of all *R. lauricola*-inoculated camphortree saplings varied from a light brown discoloration scattered throughout the xylem to a darker brown discoloration that was more continuous and uniformly distributed (Fig. 2c). Samples were obtained from the stem and branches at points at least 30 cm above the highest inoculation point, and *R. lauricola* was isolated from all inoculated camphortree saplings except one. *Raffaelea lauricola* was reisolated from xylem around the uppermost inoculation point of all inoculated camphortree saplings. Control plants appeared healthy with no evidence of wilt or discoloration, and the fungus was not isolated. All inoculated redbay saplings wilted, a dark black discoloration was observed in the xylem (Fig. 2c), and *R. lauricola* was reisolated from all inoculated plants.

3.2 Field inoculation study

All camphortree trees inoculated with *R. lauricola* at Waycross remained healthy-appearing with no evidence of wilt or branch dieback on any of the evaluation dates. However, when the trees were destructively sampled, a brown discoloration

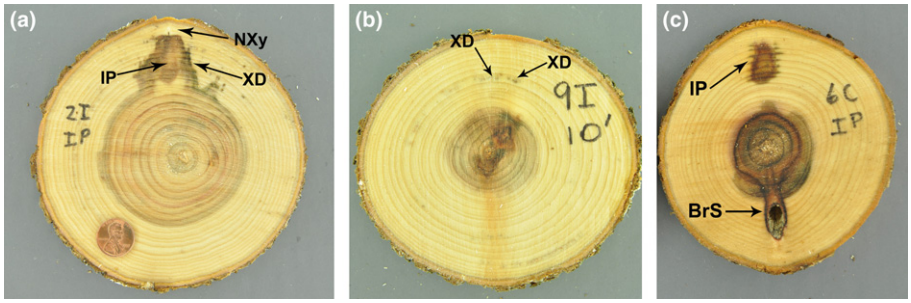

Fig. 3. Discs cut from the stems of camphortrees at Waycross, Georgia in June 2012, 62 weeks after wounding and inoculation with *Raffaelea lauricola* or sterile malt extract agar. (a) Xylem discoloration (XD) around the inoculation point (IP) of tree W2I, inoculated with *R. lauricola*. An area of new xylem (NXy) laid down after inoculation; (b) points of xylem discoloration at 1.7 m above the inoculation point on tree W9I after inoculation with *R. lauricola*; (c) discoloration at the inoculation point (IP) of tree W6C, mock-inoculated with a plug of sterile malt extract agar. Also present is an overgrown branch stub (BrS).

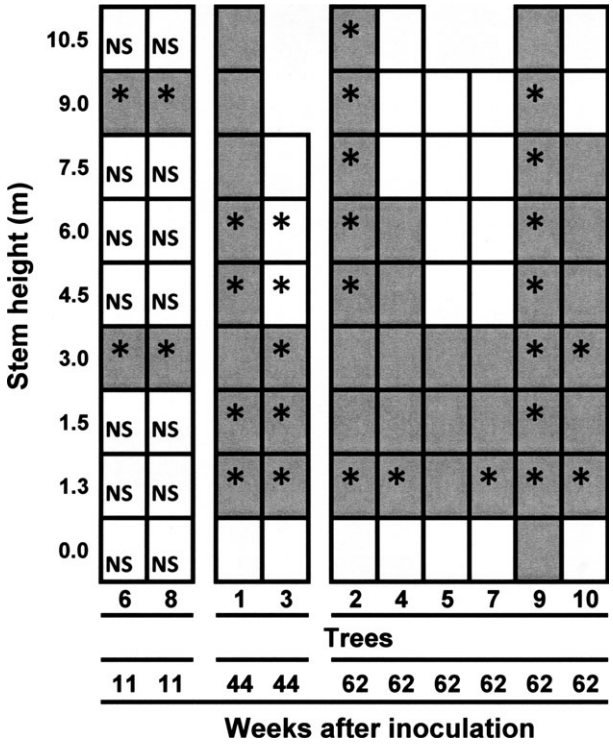

Fig. 4. Presence of xylem discoloration (grey shading) and isolation of *Raffaelea lauricola* (asterisk) from 10 inoculated camphortree trees at various stem heights at 11, 44 and 62 weeks following inoculation with *R. lauricola* at 1.3 m height. Discoloration was noted and isolations were attempted at only 3.0 and 9.0 m for the first sampled trees at 11 weeks after inoculation (other heights are designated as NS). Rectangles not outlined were above the height of the trees at the time of sampling.

was observed in the xylem around inoculation points and in patchy areas at varying heights in many trees, and *R. lauricola* was reisolated (Figs 3 and 4). Two trees felled 2 months after inoculation had areas of light discoloration in the xylem at 3 and 9 m height, and *R. lauricola* was reisolated from the discoloured areas. The remaining camphortrees harvested at 10 or 14 months were found to have areas of dark discoloration around the inoculation points, and *R. lauricola* was reisolated from seven of the eight trees. *Raffaelea lauricola* was not isolated at any point above the inoculation point in trees W4, W5 and W7, although some discs from these trees exhibited areas of light discoloration at different heights. In trees W1, W2, W3, W9 and W10, *R. lauricola* was reisolated at various heights above the inoculation point, but not all plated xylem samples yielded the fungus. For instance, *R. lauricola* was isolated from a disc at 3 m height in tree W10 but was not isolated from the other discs despite the presence of a light, patchy discoloration in some discs. *R. lauricola* was isolated from discs obtained at heights up to 10.5 m in tree W2 and 9 m in tree W9, although the fungus was not reisolated from some discs taken at lower heights from these trees. *Raffaelea lauricola* was isolated from discs at heights of 4.5 and 6.0 m in tree W3, although areas of discoloration were not distinct in the sapwood of these discs. There was no sapwood discoloration, and

R. lauricola was not isolated from below the inoculation point of any tree except W9. Areas of discoloration were not observed in the xylem of control trees, except for the immediate vicinity of the wound, and *R. lauricola* was not isolated.

Isolates of *R. lauricola* that were reisolated from camphortrees at 10 or 14 months were later shown to be aggressive on redbay. Symptoms were observed in some redbay saplings within 10 days after inoculation. By day 28, the foliage of all saplings had completely wilted and discoloration was observed in the xylem.

3.3 Association of *Xyleborus glabratus* and *Raffaelea lauricola* with camphortrees

Short beetle tunnels similar in diameter to those made by *X. glabratus* were common in the sapwood of camphortrees on Jekyll Island (Table 1). The tunnels were more numerous in the sapwood of trees that were exhibiting branch dieback (mean = 24.6 tunnels per tree) than those that appeared healthy (mean = 4.8; $p < 0.006$). Most tunnels had no adult beetles or other insect life stages. The tunnels were simple, with no branching, and extended into the xylem only 0.5–3.0 cm. Some of the attacks appeared to be recent, but others were overgrown with 1–4 years of new xylem and phloem (Fig. 5c,d). Xylem discoloration in transverse sections was limited to areas immediately around beetle tunnels; however, in longitudinal sections, streaks of dark brown discoloration extended many centimetres above and below the beetle tunnels (Fig. 5a–c).

Raffaelea lauricola was frequently isolated from areas of discoloration in all trees that exhibited dieback and was also isolated from four of the five healthy-appearing trees, but the isolation frequencies were greater from trees that had branch dieback (Table 1). Isolation frequencies of *R. lauricola* were only slightly greater from discoloration near the beetle tunnels than from discoloration not near the beetle tunnels (Table 1).

No successful brood galleries were seen, but adult, female *X. glabratus* were found in two of the trees with branch dieback. In tree J7, 31 beetle entrance holes were found, and most of the attacks were recent (i.e. entrance holes were not overgrown with new xylem and phloem). *R. lauricola* was isolated from xylem around each of the 14 beetle tunnels that were sampled and plated on CSMA (Table 1). Seven of the tunnels in tree J7 were found to contain a single, live female *X. glabratus*. In tree J10, 33 beetle entrance holes were found, but most of the entrance holes were overgrown and had been initiated 1 or 2 years prior to observation. A single dead, female adult *X. glabratus* was found in each of three tunnels in J10. *Raffaelea lauricola* was isolated from the xylem around 32 of the 33 tunnels in J10 (Table 1).

Tree J6 had branch mortality and 35 beetle entrance holes, but many of these were probably due to the black twig beetle, *Xylosandrus compactus*. Of 17 beetle tunnels examined, 11 tunnels had enlarged chambers typical of those created by *X. compactus*, and adult *X. compactus* beetles were found in three of the tunnels. One of the beetle tunnels with *X. compactus* adults was from a 5-cm-diameter stem section at 90 cm above the ground. Dark brown sapwood discoloration was found around the tunnels, similar to the discoloration around tunnels formed by *X. glabratus* (Fig. 5b). However, *R. lauricola* was isolated from only 16% of the samples taken from discoloured sapwood around beetle tunnels in tree J6. Because of difficulties distinguishing the tunnels made by *X. glabratus* and *X. compactus*, the results from tree J6 are not included in Table 1.

Table 1. Beetle entrance holes typical of those made by *Xyleborus glabratus*, number of tunnels with *X. glabratus* adults, and isolation of *Raffaelea lauricola* from samples with discoloured sapwood near and away from tunnels in stems of healthy-appearing camphortrees and trees with branch dieback on Jekyll Island, Georgia.

Tree[1]	Date	Tree condition	Height (m)	DBH (cm)	Beetle entrance holes (no.)	Tunnels with *X. glabratus* (no.)	Isolation of *R. lauricola* from discoloured sapwood (percentage positive samples (no. evaluated))	
							Around tunnels	Not near tunnels
J3	13 May 2011	Healthy	10.8	9.5	5	0	80 (5)	25 (4)
J4	9 Feb 2012	Healthy	9.6	6.5	11	0	25 (8)	66 (29)
J5	9 Feb 2012	Healthy	8.5	6.4	5	0	0 (4)	0 (3)
J8	12 Apr 2012	Healthy	10.0	6.4	4	0	75 (4)	94 (18)
J9	12 Apr 2012	Healthy	11.6	8.9	1	0	100 (1)	NS[5]
Means			10.1a[2]	7.5a	5.2a	0	56	46.2
J1	15 Oct 2010	Dieback	7.0	8.9	5	0	100 (5)	100 (5)
J2	7 Apr 2011	Dieback	9.3	10.2	25	0	91 (22)	62 (8)
J7	12 Apr 2012	Dieback	10.2	8.0	31	7[3]	100 (14)	100 (11)
J10	12 Apr 2012	Dieback	8.8	9.9	33	3[4]	97 (33)	96 (23)
Means			8.8a	9.3a	23.5b	2.5	97	89.5

[1]Data for dieback tree J6 not shown because beetle galleries were typical for *Xylosandrus compactus* and *R. lauricola* were isolated from only 16% of the discoloured areas around beetle tunnels.
[2]Means within column followed by the same letter do not differ significantly ($p > 0.05$).
[3]All beetles were alive.
[4]All beetles were dead.
[5]No samples evaluated.

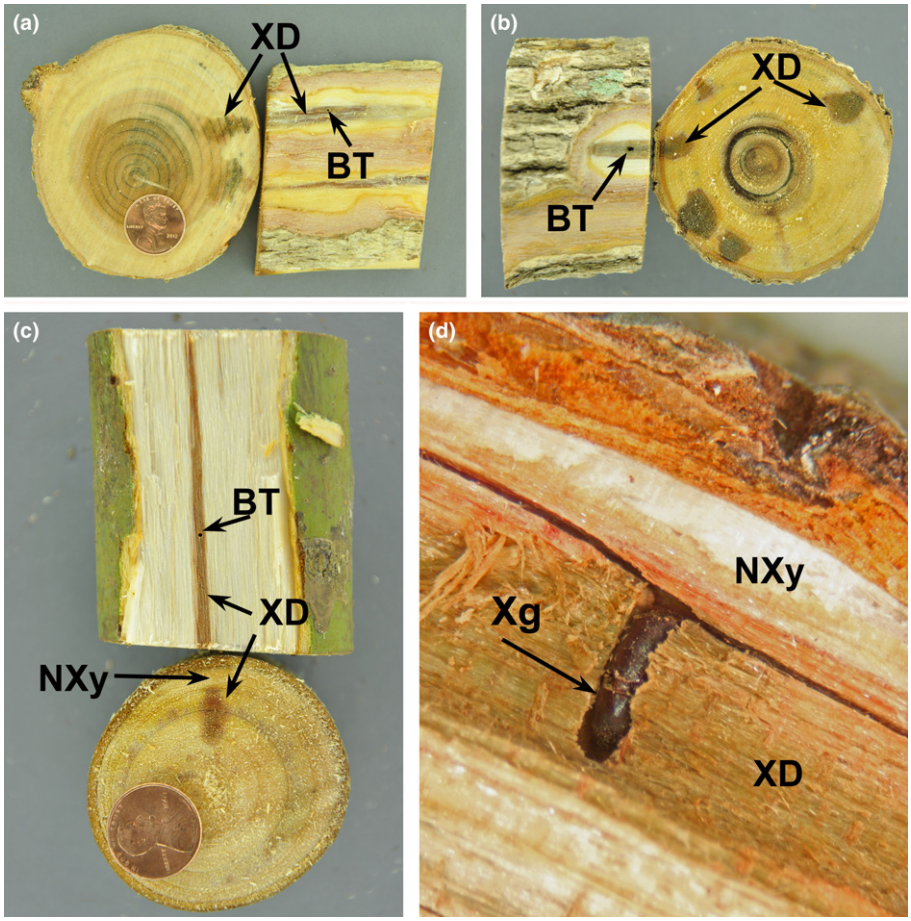

Fig. 5. Discs cut from the stems of camphortrees with branch dieback at Jekyll Island, Georgia. (a) Beetle tunnel (BT) located at site of beetle entrance hole, and xylem discoloration (XD) typically associated with attacks by *Xyleborus glabratus* and from which *Raffaelea lauricola* was readily isolated; (b) beetle tunnel and areas of xylem discoloration in tree J6 from which *Xylosandrus compactus* was found, but *R. lauricola* was not recovered (c) xylem discoloration and beetle tunnel in longitudinal section above, and in transverse section with xylem discoloration associated with beetle tunnel overgrown with new xylem (NXy); (d) beetle tunnel with dead *X. glabratus* (Xg) adult female surrounded by discoloured xylem and tunnel entrance overgrown with new xylem.

Insect tunnels were also found in twigs and small diameter branches (range 7–30 mm diameter) in the crowns of trees J2 and J10, which had branch dieback, and trees J3, J8 and J9, which did not have branch dieback. In some cases, the insect holes were present in dead twigs or small diameter branches, and in other cases, portions of the small diameter branches were still alive but had a dark brown to black discoloration in the sapwood. Some of these tunnels may have been made by *X. glabratus*, but many of the tunnels in twigs and small diameter branches were attributed to other species of ambrosia beetles. Thirty insect tunnels in twigs and small diameter branches were examined, and nine had adult *X. compactus.* Eight others were abandoned but were thought to be due to *X. compactus* based on the presence of tunnels with enlarged chambers, which are typical for this ambrosia beetle. Of the 30 examined twigs and small branches, 11 were from tree J2. The beetle damage in ten of these J2 samples was of unknown origin, but a dead adult *Xyleborinus saxeseni* was found in one of the tunnels. Samples of discoloured xylem near eight of the tunnels in tree J2 and discoloured xylem near six tunnels in other trees were plated on CSMA, and all were negative for *R. lauricola*.

Eight fungal isolates from camphortrees on Jekyll Island, tentatively identified as *R. lauricola* based on morphological characteristics, were subsequently confirmed as *R. lauricola* based on sequencing of a portion of the LSU. Redbay saplings inoculated with the *R. lauricola* isolates developed xylem discoloration and wilted. Loss of turgor in leaves and discoloration in leaf veins were first observed in some plants within 12 days after inoculation. Most saplings exhibited wilt symptoms within 16 days, and by day 27, all saplings died. *Raffaelea lauricola* was reisolated from all inoculated plants. Redbay plants used as controls remained healthy, and *R. lauricola* was not isolated.

4 Discussion

Camphortree is susceptible to laurel wilt, but it is much less susceptible compared to the species of Lauraceae indigenous to the southeastern United States. In the American species, a single inoculation with *R. lauricola* on the stem of a sapling or tree, or an attack by a single *X. glabratus* female, is sufficient to infect plants, and mortality generally follows within

6–12 weeks (Fraedrich et al. 2008, 2011; Mayfield et al. 2008). In contrast, a single inoculation point with *R. lauricola* on camphortree saplings or trees failed to cause wilt symptoms or branch dieback in the test plants. However, multiple inoculation points along the stem of saplings induced wilt-like symptoms and branch dieback. Movement of the pathogen in camphortree was demonstrated with saplings and mature trees inoculated at a single point, but systemic colonization did not appear to be as rapid or extensive in camphortree as it was in two American species, redbay and avocado.

On Jekyll Island, where laurel wilt has been severe on redbay, mortality from laurel wilt has not been observed in camphortrees, but the 10 trees examined in this study had evidence of attacks by *X. glabratus,* and nine of the trees were naturally infected with *R. lauricola*. Four of the five sampled camphortrees with branch dieback showed evidence of multiple attacks by *X. glabratus* that appeared to have occurred over several years, and *R. lauricola* was frequently recovered from discoloured xylem near the beetle tunnels and further away. *Raffaelea lauricola* has been previously documented in camphortrees with shoot and branch dieback in the southeastern United States, but neither *X. glabratus* adults nor beetle entrance holes similar in size to those produced by *X. glabratus* were observed in those trees (Smith et al. 2009). In the present study, *X. glabratus* adults were found in tunnels of two of the 10 camphortrees examined at Jekyll Island, and the diameter of most of the apparently abandoned tunnels in these and other camphortrees was typical of those made by *X. glabratus*. *Xyleborus glabratus* is the only common vector of *R. lauricola* (Harrington and Fraedrich 2010), and the pathogen was regularly isolated from the discoloured sapwood around beetle tunnels in nine of the 10 trees examined. It is most likely that these infections were the result of aborted attacks by *X. glabratus.*

Multiple attacks by *X. glabratus* and the associated infections by *R. lauricola* may be necessary to cause branch dieback in camphortrees. In this regard, the dieback observed in camphortrees may be similar to the dieback and wilt-like symptoms of indigenous oaks in Japan and Korea associated with multiple attacks by ambrosia beetles in the genus *Platypus,* whose symbionts are also *Raffaelea* spp. (Esaki et al. 2004; Murata et al. 2007; Lee et al. 2011). The *Raffaelea* spp. involved in the dieback and mortality of the Asian oak species do not function as systemic, vascular wilt pathogens, but instead cause localized lesions and blockages in the sapwood around beetle tunnels (Murata et al. 2009; Lee et al. 2011). Vertical and horizontal movement of the *Raffaelea* spp. and loss of water conductance are confined to areas immediately around the attacks, and thousands of attacks by the *Platypus* spp. ultimately lead to dieback and mortality in these oak trees (Takahashi et al. 2010). Although *R. lauricola* moves vertically in the vascular system of camphortree, horizontal movement of the fungus appears to be restricted. Small patches of light to dark brown discoloration were scattered throughout the xylem, but the intense discoloration that occurs in the outermost sapwood rings of infected redbay was not observed in inoculated camphortrees or in naturally infected camphortrees with branch dieback. Branch dieback was most common and sapwood discoloration most abundant in the camphortrees that had the greatest number of beetle tunnels in the sapwood of the main stems, and *R. lauricola* was isolated from 90% of the discoloured areas located away from beetle tunnels. Nonetheless, even the healthy-appearing trees had some aborted beetle attacks and the pathogen was frequently isolated from discoloured sapwood, suggesting that *X. glabratus* routinely attacks even healthy camphortrees, at least in areas with high population levels of *X. glabratus*.

The association of *X. glabratus* or *R. lauricola* with branch dieback or wilt of camphortree has not been reported in eastern Asia where the tree, beetle and fungus have coevolved. However, the disease may be endemic there, occurring at low levels and associated with stressed and injured camphortrees, a situation possibly analogous to the association of *Ophiostoma himal-ulmi* with *Ulmus* spp. that are indigenous to the Himalayas. There *O. himal-ulmi* is associated with wounds on elms caused by an endemic bark beetle, *Scolytus kashmirensis* (Brasier and Mehrotra 1995; Buhroo 2012). Like the Dutch elm disease pathogens, *O. ulmi* and *O. novo-ulmi*, *O. himal-ulmi* is systemic and highly pathogenic in inoculations of European elms, but disease symptoms have not been found in *U. wallichiana* or other elms native to the Himalayas (Brasier and Mehrotra 1995). *Ophiostoma himal–ulmi* and *S. kashmirensis* are thought to be primarily associated with wounded, stressed and declining elm trees (Brasier and Mehrotra 1995; Buhroo 2012). Similarly, *R. lauricola* and *X. glabratus* may occupy a similar niche within their native range, perhaps attacking weakened and dying camphortrees and other suitable tree species. The strong attraction of the *X. glabratus* to host volatiles, rather than plant fermentation products like ethanol (Hanula and Sullivan 2008; Kendra et al. 2012), may indicate that the *X. glabratus*/*R. lauricola* symbiosis in Asia depends on the beetle introducing the plant pathogen to live trees during inoculation attacks, to be followed by systemic colonization by the fungus and subsequent brood-laying attacks in diseased branches or stems (Harrington et al. 2011).

Xylosandrus compactus is an exotic ambrosia beetle that was introduced into the southeastern United States from Asia in the early 1940s and is a pest of many woody plants, typically attacking live twigs and small branches less than 2 cm diameter (Ngoan et al. 1976; Dixon and Woodruff 1982). In the present study, we observed *X. compactus* beetles, larvae and eggs in tunnels in a stem of camphortree that was 5 cm diameter, which suggests that this beetle is not restricted to smaller diameter twigs and branches as commonly reported. In trees where *X. compactus* beetles or *X. compactus*-like tunnels were commonly observed, *R. lauricola* was recovered from only 25% or less of the samples of discoloured sapwood around beetle tunnels. Although we (T. C. Harrington and S. W. Fraedrich, unpublished data) and others (von Arx and Hennebert 1965; Batra 1967) have found *A. xylebori* to be the mycangial symbiont of *X. compactus*, *Fusarium solani* and a *Botryosphaeria* sp. have been associated with xylem discoloration in twigs and branches attacked by *X. compactus (*Wilson et al. 2005; Fraedrich et al. 2011). Additional research is needed to determine the species of fungi associated with sapwood staining caused by *X. compactus* in camphortree and to determine whether these fungi are also involved in the dieback that is occasionally observed in this tree species.

The hypothesized disease cycle for laurel wilt (Fraedrich et al. 2008) starts with the attraction of *X. glabratus* adult females to volatiles from healthy hosts. These initial attacks in healthy trees are abandoned, but *R. lauricola* grows out from the beetle's mycangia and is introduced into the aborted tunnels. Tree infection and systemic movement of the fungus in

the sapwood lead to wilted trees, which are then suitable for brood production. For highly susceptible species such as redbay, it is at this time that the wilted trees are repeatedly attacked by *X. glabratus*. The results of the present study indicate that *X. glabratus* attacks healthy camphortrees and suggests that *R. lauricola* grows systemically from these aborted attacks, but we found no brood production in these stems. However, in contrast to redbay, which are completely wilted and well-colonized by *R. lauricola* at the time of egg-laying attacks by *X. glabratus*, the camphortrees in the present study were only partially affected by the disease (i.e. showed limited dieback in some trees), and stem discoloration and colonization by *R. lauricola* were very patchy. Thus, the stems of the camphortrees may not have been suitable at this time for egg-laying attacks by *X. glabratus*. Other studies have found that camphortree bolts were highly attractive to *X. glabratus*, and the beetles attacked the bolts, but emergence of brood from the bolts was very low (Mayfield and Hanula 2012, S. W. Fraedrich unpublished data). The bolts used in these studies were from healthy camphortrees that were not colonized by *R. lauricola*. It is possible that precolonization of stem tissues by *R. lauricola* contributes to the successful production of brood by *X. glabratus;* however, additional studies are necessary to formally test this hypothesis.

It is likely that natural selection for resistance to *X. glabratus* and *R. lauricola* has occurred in camphortree and other Asian Lauraceae. Factors such as the rate at which tyloses are produced (Jacobi and MacDonald 1980) and vessel diameter size (Solla and Gil 2002) have been linked with resistance to other vascular wilt diseases and could be involved in the apparent resistance of camphortrees to laurel wilt. Induced and constitutive chemical compounds found in camphortrees (Shi et al. 1989; Pelissier et al. 1995; Boulogne et al. 2012) could also have a role in protecting trees from *X. glabratus* and in restricting the growth, spread and survival of *R. lauricola* within trees.

References

von Arx, J. A.; Hennebert, G. L., 1965: Deux champignons ambrosia. Mycopathol. Mycol. Appl. **25**, 309–325.

Batra, L. R., 1967: Ambrosia fungi: a taxonomic revision and nutritional studies of some species. Mycologia **59**, 976–1017.

Boulogne, I.; Petit, P.; Ozier-Lafontaine, H.; Desfontaines, L.; Loranger-Merciris, G., 2012: Insecticidal and antifungal chemicals produced by plants: a review. Environ. Chem. Lett. **10**, 325–347.

Brasier, C. M.; Mehrotra, M. D., 1995: *Ophiostoma himal-ulmi* sp. nov., a new species of Dutch elm disease fungus endemic to the Himalayas. Mycol. Res. **99**, 205–215.

Buhroo, A. A., 2012: Host selection behavior and incidence of the bark beetle *Scolytus kashmirensis* (Coleoptera: Curculionidae: Scolytinae) attacking elm (*Ulmus* spp.) trees in Kashmir. For. Stud. China **14**, 224–228.

Dixon, W. N.; Woodruff, R. E., 1982: The black twig borer, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Scolytidae). Fla. Dep. Agric. Consum. Serv., Div. Plant Ind., Entomol. Circ. 250. Gainesville, FL.

Esaki, K.; Kato, K.; Kamata, N., 2004: Stand-level distribution and movement of *Platypus quercivorus* adults and patterns of incidence of new infestation. Agric. For. Entomol. **6**, 71–82.

Fraedrich, S. W.; Harrington, T. C.; Rabaglia, R. J.; Ulyshen, M. D.; Mayfield, A. E. III.; Hanula, J. L.; Eickwort, J. M.; Miller, D. R., 2008: A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Dis. **92**, 215–224.

Fraedrich, S. W.; Harrington, T. C.; Bates, C. A.; Johnson, J.; Reid, L. S.; Best, G. S.; Leininger, T. D.; Hawkins, T. S., 2011: Susceptibility to laurel wilt and disease incidence in two rare plant species, pondberry and pondspice. Plant Dis. **95**, 1056–1062.

Hanula, J. L.; Sullivan, B., 2008: Manuka oil and phoebe oil are attractive baits for *Xyleborus glabratus* (Coleoptera Scolytinae), the vector of laurel wilt. Environ. Entomol. **37**, 1403–1409.

Hanula, J. L.; Mayfield, A. E. III.; Fraedrich, S. W.; Rabaglia, R. J., 2008: Biology and host associations of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae), exotic vector of laurel wilt killing redbay trees in the southeastern United States. J. Econ. Entomol. **101**, 1276–1286.

Hanula, J. L.; Ulyshen, M. D.; Horn, S., 2011: Effect of trap type, trap position, time of year, and beetle density on captures of the redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. **104**, 501–508.

Harrington, T. C., 1981: Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. Mycologia **73**, 1123–1129.

Harrington, T. C., 1992: Leptographium. In: Methods for Research on Soilborne Phytopathogenic Fungi. Ed. by Singleton, L. L.; Mihail, J. D.; Rush, C. M. St. Paul, MN: American Phytopathological Society, pp. 129–133.

Harrington, T. C.; Fraedrich, S. W., 2010: Quantification of propagules of the laurel wilt fungus and other mycangial fungi from the redbay ambrosia beetle, *Xyleborus glabratus*. Phytopathology **100**, 1118–1123.

Harrington, T. C.; Fraedrich, S. W.; Aghayeva, D. N., 2008: *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. Mycotaxon **104**, 399–404.

Harrington, T. C.; Aghayeva, D. N.; Fraedrich, S. W., 2010: New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella* and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. Mycotaxon **111**, 337–367.

Harrington, T. C.; Yun, H. Y.; Lu, S. S.; Goto, H.; Aghayeva, D. N.; Fraedrich, S. W., 2011: Isolations from the redbay ambrosia beetle, *Xyleborus glabratus*, confirm that the laurel wilt pathogen, *Raffaelea lauricola*, originated in Asia. Mycologia **103**, 1028–1036.

Hughes, M. A.; Shin, K.; Eickwort, J.; Smith, J. A., 2012: First report of laurel wilt disease caused by *Raffaelea lauricola* on silk bay in Florida. Plant Dis. **96**, 910–911.

Hulcr, J.; Lou, Q.-Z., 2013: The redbay ambrosia beetle (Coleoptera: Curculionidae) prefers Lauraceae in its native range: records form the Chinese National Insect Collection. Fla. Entomol. **96**, 1595–1597.

Inch, S. A.; Ploetz, R. C., 2012: Impact of laurel wilt, caused by *Raffaelea lauricola*, on xylem function in avocado, *Persea americana*. For. Path. **42**, 239–245.

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

Jacobi, W. R.; MacDonald, W. L., 1980: Colonization of resistant and susceptible oaks by *Ceratocystis fagacearum*. Phytopathology **70**, 618–623.

Kendra, P. E.; Niogret, J.; Montgomery, W. S.; Sanchez, J. S.; Deyrup, M. A.; Pruett, G. E.; Ploetz, R. C.; Epsky, N. D.; Heath, R. R., 2012: Temporal analysis of sesquiterpene emissions from manuka and phoebe oil lures and efficacy for attraction of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. **105**, 659–669.

Langeland, K. A.; Cherry, H. M.; McCormick, C. M.; Craddock Burks, K. A., 2008: Identification and Biology of Nonnative Plants in Florida's Natural Areas. Gainesville, FL: University of Florida, IFAS Communication Services.

Lee, J. S.; Haack, R. A.; Choi, W. I., 2011: Attack pattern of *Platypus koryoensis* (Coleoptera: Curculionidae: Platypodinae) in relation to crown dieback of Mongolian oak in Korea. Environ. Entomol. **40**, 1363–1369.

Mayfield, A. E. III.; Hanula, J. L., 2012: Effect of tree species and end seal on attractiveness and utility of cut bolts to the redbay ambrosia beetle and granulate ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. **105**, 461–470.

Mayfield, A. E. III.; Barnard, E. L.; Smith, J. A.; Bernick, S. C.; Eickwort, J. M.; Dreaden, T. J., 2008: Effect of propiconazole on laurel wilt disease development in redbay trees and on the pathogen in vitro. Arboric. Urban For. **34**, 317–324.

Murata, M.; Yamada, T.; Matsuda, Y.; Ito, S., 2007: Discoloured and non-conductive sapwood among six Fagaceae species inoculated with *Raffaelea quercivora*. For. Path. **37**, 73–79.

Murata, M.; Matsuda, Y.; Yamada, T.; Ito, S., 2009: Differential spread of discoloured and non-conductive sapwood among four Fagaceae species inoculated with *Raffaelea quercivora*. For. Path. **39**, 192–199.

Ngoan, N. D.; Wilkinson, R. C.; Short, D. E.; Moses, C. S.; Mangold, J. R., 1976: Biology of an introduced ambrosia beetle, *Xylosandrus compactus*, (Coleoptera-Scolytidae) in Florida. Ann. Entomol. Soc. Am. **69**, 872–876.

Pelissier, Y.; Marion, C.; Prunac, S.; Bessiere, J., 1995: Volatile components of leaves, stems and bark of *Cinnamomum camphora* Nees et Ebermaier. J. Essent. Oil Res. **7**, 313–315.

Ploetz, R. C.; Perez-Martinez, J. M.; Smith, J. A.; Hughes, M.; Dreaden, T. J.; Inch, S. A.; Fu, Y., 2012: Responses of avocado to laurel wilt, caused by *Raffaelea lauricola*. Plant. Pathol. **61**, 801–808.

Shi, W. Y.; Wei, H.; Wen, G. Y.; Guo, D. X.; Long, G. Y.; Liu, Y. G., 1989: Study on chemical constituents of the essential oil and classification of types from *Cinnamomum camphora*. Acta Bot. Sin. **31**, 209–214.

Smith, J. A.; Mount, L.; Mayfield, A. E. III.; Bates, C. A.; Lamborn, W. A.; Fraedrich, S. W., 2009: First report of laurel wilt disease caused by *Raffaelea lauricola* on camphor in Florida and Georgia. Plant Dis. **93**, 198.

Solla, A.; Gil, L., 2002: Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. For. Path. **32**, 123–134.

Steel, R. G. D.; Torrie, J. H., 1980: Principles and Procedures of Statistics: A Biometrical Approach. New York, NY: McGraw-Hill.

Takahashi, Y.; Matsushita, N.; Hogetsu, T., 2010: Spatial distribution of *Raffaelea quercivora* in xylem of naturally infested and inoculated oak trees. Phytopathology **100**, 747–755.

Wilson, A.; Schiff, N.; Leininger, T.; Hamel, P.; Gardiner, E.; Connor, K.; Devall, M., 2005: Black twig borer exacerbates damage caused by Botryosphaeria stem canker and dieback of pondberry in the Delta National Forest of Mississippi. Phytopathology **95**, S112.

Wood, S. L., 1982: The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae), A Taxonomic Monograph. Great Basin Naturalist Memoirs. No. 6. Provo, UT: Brigham Young University.

Wood, S. L.; Bright, D. E., 1992: A Catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index, Volumes A and B. Great Basin Naturalist Memoirs No. 13. Provo, UT: Brigham Young University.