Raffaelea lauricola and *Xyleborus glabratus* Cause Laurel Wilt, A Lethal Disease *of* Redbay (*Persea borbonia*) and Other Species in the Lauraceae

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BACKGROUND INFORMATION

Extensive mortality of redbay (*Persea borbonia* (L.) Spreng) has been observed in the coastal plains of South Carolina and Georgia since 2003. The disease was subsequently observed in northeast Florida in 2005 and has spread rapidly in that state. The current range of the disease is presented in Figure 1.

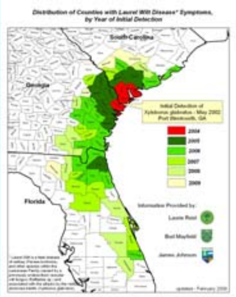

Figure 1. Range of laurel wilt, February, 2009.

Dead and dying trees exhibit wilt-like symptoms (Figure 2). Trees often decline very rapidly with the wilt affecting the entire crown uniformly. In other cases, tree decline progresses more slowly affecting individual branches one at a time. The sapwood of the main stem and branches of affected trees exhibits discoloration (Figure 3). Small beetle entrance holes and tunnels are normally found in association with discolored areas of the sapwood. On some symptomatic trees the entrance holes have been rare and difficult to locate. On other trees, entrance holes are numerous.

The redbay ambrosia beetle, *Xyleborus glabratus (*Eichhoff), has been routinely obtained from dead and dying trees. This non-native beetle was first trapped in the United States in 2002 at a port facility near Savannah GA. The recovery of the beetle from dead and dying redbay on Hilton Head Island (SC) in November, 2004 was the first indication that the beetle was established in forests of the southeastern USA. The beetle is native to Asia (e.g. India, Japan) where it is associated with plant species in the family Lauraceae (e. g. *Lindera latifolia, Litsea elongata).*

Figure 3. Sapwood discoloration in a dying redbay tree near Colonels Island, GA (May, 2005).

A fungus was consistently isolated from the discolored xylem of symptomatic trees throughout the range of the problem. Initially the fungus was identified as an *Ophiostoma* sp. based on sequences of the ribosomal DNA and its tolerance of cycloheximide. The anamorphic stage of the fungus was subsequently identified as a new species of *Raffaelea*, *R. lauricola*. The fungus is a symbiont of *X. glabratus* and is located in the mycangia that are located behind the mandibles of the beetles.

During the spring and summer of 2005, sassafras (*Sassafras albidum*) mortality was also observed at several locations in coastal counties of Georgia. An examination of the trees found sapwood discoloration similar to that observed in redbay. *Xyleborus glabratus* and *R. lauricola* have been routinely isolated from the wilted sassafras.

PATHOGENICITY TESTS

The pathogenicity of isolates of *R. lauricola* to redbay was evaluated in growth chamber and field tests. The field test was conducted in March, 2005 near Bluffton, SC. Twenty-four redbay trees (heights, 1.8 to 4.9 m) were selected and paired. All trees were wounded (i.e. drilled holes). One tree of each pair was inoculated with an isolate of *R. lauricola*.; the other tree was treated as a control. Trees were evaluated 10 weeks after inoculation. All trees inoculated with *R. lauricola* wilted and exhibited sapwood discoloration (Figure 4 A, B). Three of the 12 control trees also wilted but these trees had beetle entrance holes characteristic of *X. glabratus* as well as sapwood discoloration. The other nine control trees were healthy with no evidence of sapwood discoloration or entrance holes. Pieces of sapwood from all trees were placed on cycloheximide -streptomycin malt agar (CSMA) medium, and *R. lauricola* was isolated from discolored sapwood of all wilted control and fungus-inoculated trees.

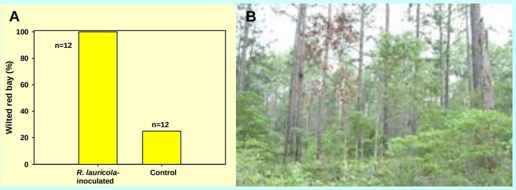

Figure 4. A) Percentage of wilted redbay trees following inoculation with *R. lauricola*; B) Wilted, *R. lauricola*-inoculated redbay (left) and healthy, control tree (right).

Figure 2. (A) Wilted redbay at Jekyll Island, GA (October, 2006) and (B) at Fort George Island near Jacksonville, FL (October, 2005)

PATHOGENICITY TESTS, continued

In the growth chamber test, plants were wounded and inoculated with conidial suspensions of *R. lauricola* isolates. Control plants were wounded and drops of sterile water were placed at the wound site. Plants were placed in a growth chamber (16 h photoperiod; temperatures, 25 to 28°C). Eight weeks after inoculation 19 of 20 plants inoculated with *R. lauricola* exhibited symptoms of the wilt (Figs. 5 A, B). All wilted plants had sapwood discoloration, and *R. lauricola* was isolated from all symptomatic plants. Control plants remained healthy and exhibited no symptoms of disease.

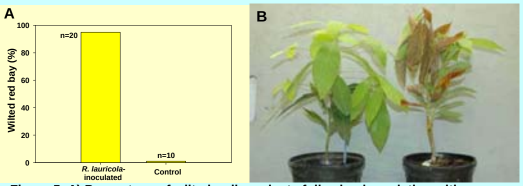

Figure 5. A) Percentage of wilted redbay plants following inoculation with isolates of *R. lauricola*; B) Control plant (left) and wilted, *Raffaelea*-inoculated redbay plant (right).

REDBAY PLANTS CHALLENGED WITH *XYLEBORUS GLABRATUS*

The objective of this experiment was to determine if *X. glabratus* beetles could attack healthy redbay seedlings. Beetles were obtained from infested redbay stems, placed in gel caps, and the caps were attached to the stems of the plants (Figure 6A). Most beetles bored into the seedlings within 48 hours (Figure 6B). The seedlings were observed over the course of the next eight weeks. Sixteen of the 22 seedlings, attacked by *X. glabratus,* wilted and exhibited sapwood discoloration (Figure 7A, B) *Raffaelea lauricola* was isolated from all symptomatic plants as well as from five of the nonsymptomatic seedlings. The fungus was isolated only around beetle tunnels in the nonsymptomatic plants.

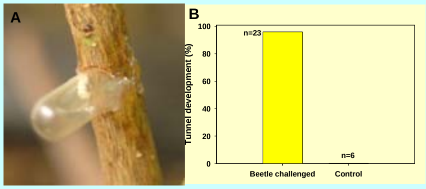

Figure 6. A) Gel cap with beetle attached to redbay plant (note wood boring dust in cap); B) Percentage of redbay plants with tunnel development after being challenged with *X. glabratus.*

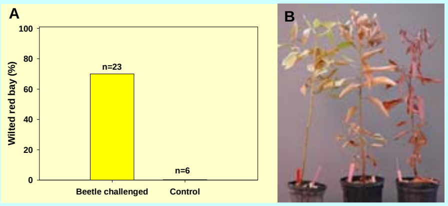

Figure 7. A) Percentage wilted redbay plants following colonization by *X. glabratus*; B) Symptomatic redbay plants attacked by *X. glabratus* and subsequently infected with *R. lauricola*.

SUSCEPTIBILITY OF OTHER SPECIES IN THE LAURACEAE

The susceptibility of sassafras (*Sassafras albidum*), spicebush (*Lindera benzoin*) and swampbay (*P. borbonia var. pubescens*) to wilt caused by *R. lauricola* was also evaluated. Redbay plants were also inoculated for comparison, and all plants were grown in 1 or 3 gallon pots. Ten plants of each species were wounded and inoculated with conidial suspensions of isolates of *R. lauricola*. Five control plants of each species were also wounded and drops of sterile water were placed at wound sites. Plants were incubated in a growth chamber (16 h photoperiod; temperatures, 25 to 28°C).

Spicebush, sassafras, swampbay as well as redbay wilted following inoculation with isolates of *R. lauricola*. (Figures 8A, B). Wilted plants of all species had sapwood discoloration. In spicebush, leaf chlorosis and wilting was evident in some plants within 5 days after inoculation, and most plants were completely wilted within 3 weeks. In sassafras, leaves on inoculated plants turned red and readily abscised from stems. In redbay and swampbay, new shoots at branch terminals wilted. More mature leaves developed a reddish to purplish brown discoloration and dead leaves were persistent on plants. All redbay and swampbay plants died within 5 weeks after inoculation. *R. lauricola* was isolated on CSMA medium from the discolored sapwood of all wilted plants.

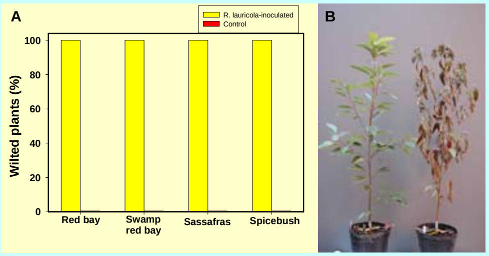

Figure 8. A) Percentage wilted redbay, swampbay, sassafras and spicebush plants following inoculation with isolates of *R. lauricola*; B) Control (left) and *R. lauricola*-inoculated (right) sassafras plants after five weeks.

SUMMARY AND CONCLUSIONS

Xyleborus glabratus and *Raffaelea lauricola* are the causes of extensive mortality of redbay and sassafras in the southeastern USA. *Xyleborus glabratus* is capable of attacking healthy plants, and *R. lauricola* causes a rapid wilt in susceptible plants.

Based on the results of these studies, and the association of *X. glabratus* with species in the Lauraceae, there is reason to be concerned that laurel wilt could affect other members of this family in the Americas. Many plant species in the Lauraceae occur in Central and South America, and are common components of forests. Increasing accounts of the wilt on sassafras, pondspice (*Litsea aestivalis*), and avocado (*P. americana*) provide support for this concern. Additional studies have shown that pondberry (*Lindera melissaefolia*) and pondspice, which are endangered or threatened species, are highly susceptible to wilt caused by *R. lauricola*. A west coast species, California laurel (*Umbellularia californica*) has also been shown to be susceptible to the wilt in laboratory studies.