Association of *Xyleborus glabratus* and an *Ophiostoma* sp. with mortality of red bay (*Persea borbonia)* in Georgia and South Carolina.

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BACKGROUND INFORMATION

Red bay (*Persea borbonia* (L.) Spreng) is an aromatic, evergreen tree or shrub that is common in forests in the Atlantic and Gulf Coastal Plains of the southeastern United States. Trees can attain heights as great as 21 m and diameters as great as 90 cm, although tree size and growth habitat varies greatly over its range. The species is important for wildlife in southern forests, and the tree is also used as an ornamental.

Since 2003, dead and dying red bay trees have been reported on Hilton Head Island, South Carolina. In November of 2004, officials estimated that 75-80% of the island's red bays had been lost. Initial reports suggested that the problem was related to droughts during the late 1990's and early 2000's followed by above average rainfall beginning in 2003.

Recent observations and surveys by the Georgia Forestry Commission, the South Carolina Forestry Commission, and the USDA Forest Service indicate mortality of red bay is common in areas to the south and west of Savannah, Georgia as well as several southern, coastal counties of South Carolina (Figure 1).

Figure 1. Current known range of red bay mortality

SIGNS and SYMPTOMS

Dead and dying trees exhibit wilt-like symptoms. Often, a tree will decline very rapidly and the disease seems to affect the entire crown uniformly. In some trees, the decline has been observed to progress more slowly affecting individual branches one at a time. Leaves on affected trees exhibit a reddish to purplish brown discoloration and are persistent on branches (Figure 2A). The sapwood of the main stem and branches of affected trees exhibits discoloration particularly in the outer xylem (Figures 2B and 2C). In the later stages of the disease the discoloration spreads throughout the cross-sectional area of the sapwood.

Small beetle entrance holes and tunnels are normally found in association with discolored areas of the sapwood. On recently attacked trees the discoloration is prominent around beetle holes and seems to spread vertically through the tree (Figures 3A, 3B, 3C). On some symptomatic trees the entrance holes have been rare and difficult to locate, but are normally found during the course of a thorough examination. On other trees, entrance holes are numerous in discolored areas of the stem (Figure 3D).

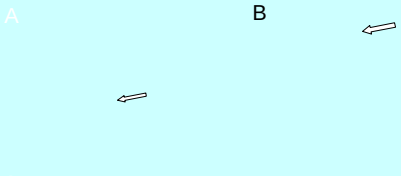

Figure 3. (A) Beetle entrance hole and sapwood discoloration at base of red bay tree; (B) Beetle entrance hole and discoloration through cross section of stem; (C) Sapwood discoloration around developing beetle gallery; (D) Sapwood discoloration on stem section with numerous beetle entrance holes.

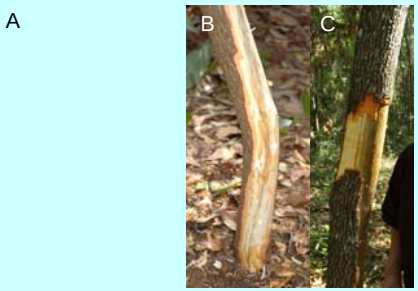

Figure 2. (A) Dead red bay near Bluffton, SC; (B, C) Sapwood discoloration on dying trees at Hilton Head, SC and south of Savannah, GA.

BEETLE AND FUNGUS ASSOCIATIONS

In November 2004, dead and dying red bay trees were examined on Hilton Head Island, SC. An ambrosia beetle was obtained from samples and was subsequently identified as *Xyleborus glabratus (*Eichhoff) (Rabaglia, personal communication). 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 samples of *P. borbonia* near Hilton Head, SC was the first indication that this beetle is established in a forest ecosystem in the United States. 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*, *Litsaea elongata*).

An anamorphic fungus was isolated from areas of sapwood discoloration on stems and branches of red bay. The fungus produces branched and unbranched conidiophores with apically formed conidia (Figure 4A). The fungus also has a yeast-like phase in which conidia produce secondary spores (Figure 4B). A preliminary DNA analysis was conducted on the fungus isolated from symptomatic red bay and it was determined to be an *Ophiostoma* sp. (Harrington, personal communication).

Subsequent evaluations of dead and dying red bay in other areas of South Carolina and Georgia have consistently found *X. glabratus* or other exotic ambrosia beetles. The anamorph of the *Ophiostoma* sp. has been consistently isolated from symptomatic sapwood.

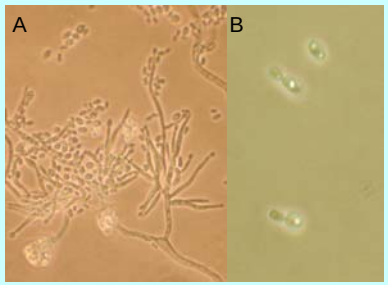

Figure 4. (A) Conidia and conidiophores of anamorph of *Ophiostoma* sp.; (B) Development of secondary spores.

Figure 5. (A) Red bay plant inoculated with an *Ophiostoma* sp. (right) and control plant (left) after 4 weeks in growth chamber pathogenicity test; (B) Red bay tree inoculated with an *Ophiostoma* sp. (right) and control tree (left) after 10 weeks.

PATHOGENICITY TESTS

Growth chamber and greenhouse. Tests were set up on May 6, 2005. In each test 15 red bay plants, grown in 1 gallon pots, were wounded by drilling holes in stems (2 mm diameter) and another 15 plants were wounded by slitting stems to a depth approximately 1/3 the diameter of the stem. Ten plants of each wound type were inoculated with drops of conidial suspensions (≈2 X 10^6 spores/ml) of one of two isolates of the *Ophiostoma* sp. The other five plants of each wound-type served as controls and drops of sterile water were placed at wound sites. One set of plants was placed in a growth chamber with a 16 h photoperiod and temperatures at 28°C (daytime) and 25°C (nighttime). The other set of plants was placed in a greenhouse where temperatures ranged from 10 to 41°C.

In the growth chamber study, symptom development was first observed within 10 days after inoculation. Leaves of fungus-inoculated plants became discolored and began to wilt, and symptoms progressed overtime to include entire branches and then entire plants (Figure 5A). Eight weeks after inoculation 19 of 20 plants exhibited symptoms of the disease, and 15 of the 20 plants were dead (Table 1). In the greenhouse study, 16 of 20 plants exhibited symptoms of the disease after 8 weeks, but only 4 fungus-inoculated plants were dead.

Field - The test was conducted in a forest near Bluffton, SC where mortality of red bay had been previously observed. Twenty-four red bay trees ranging in heights from 1.8 to 4.9 m were selected and paired based on size and proximity to one another. One tree of each pair was inoculated with one of two isolates of the *Ophiostoma* sp. on March 3 or 17, 2005; the other tree of each pair was treated as a control. A hole (5 mm diameter and 2-3 cm depth) was drilled into all trees 0.6 to 1.2 m above the groundline. Isolates of the *Ophiostoma* sp. were grown on malt extract agar and an agar plug with the fungus was inserted into the drilled holes for all fungus-inoculated trees. Seven of the control trees received a sterile agar plugs and the other five control trees received no agar plug. Inoculation points were wrapped with parafilm.

Trees were evaluated 10 weeks after inoculation. All trees inoculated with the *Ophiostoma* sp. were dead and sapwood discoloration was evident in all trees (Figure 5B). Three of the 12 control trees were also dead 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 discolored sapwood from symptomatic trees were placed on cyclohexamide-streptomycin malt agar and the imperfect stage of the *Ophiostoma* sp. was recovered from all dead control and fungus-inoculated trees.

Table 1. Pathogenicity tests for an *Ophiostoma* sp. conducted under growth chamber, greenhouse and field conditions.

	Red bay plants		
Experiment/Treatment	Inoculated	Symptomatic	Dead
Growth chamber			
Ophiostoma sp.	20	19	15
Control	10	0	0
Greenhouse			
Ophiostoma sp.	20	16	4
Control	10	0	0
Field			
Ophiostoma sp.	12	12	12
Control	12	3	3