Ability of the Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae) to Bore into Young Avocado (Lauraceae) Plants and Transmit the Laurel Wilt Pathogen (Raffaelea sp)

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ABILITY OF THE REDBAY AMBROSIA BEETLE (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE) TO BORE INTO YOUNG AVOCADO (LAURACEAE) PLANTS AND TRANSMIT THE LAUREL WILT PATHOGEN (*RAFFAELEA* SP.)

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Laurel wilt is a vascular disease of plants in the Laurel family (Lauraceae) that has caused extensive mortality of redbay (*Persea borbonia* (L.) Spreng) trees in South Carolina, Georgia, and Florida. The disease is caused by a previously undescribed species of *Raffaelea*, a fungal symbiont of the non-native redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Fraedrich et al. 2008). *Xyleborus glabratus* was initially detected in the U.S. near Savannah, GA in 2002 (Rabaglia et al. 2006). The beetle carries spores of the laurel wilt fungus in mandibular mycangia and inoculates the xylem of host trees by boring into the wood. In redbay, the fungus moves rapidly through the xylem, plugging the flow of water and causing trees to die in a matter of weeks or a few months. Affected trees are characterized by a dark discoloration in the outer sapwood (Fraedrich et al. 2008). Laurel wilt continues to devastate populations of redbay in the southeastern coastal plain and the distribution of the disease has rapidly expanded (Johnson et al. 2008).

In the summer and fall of 2006, 16 small avocado (*Persea americana* var. *americana* Mill.) trees were planted on Ft. George Island, FL, a site with a high incidence of laurel wilt. By 12 Feb 2007, *X. glabratus* and its associated *Raffaelea* sp. were obtained from two dying trees of the West Indian cultivar Donnie (A.E.M., J.E.P. & J.H.C., unpublished data). In September 2007, a large avocado tree in a homeowner's yard in Jacksonville, FL also was discovered to be diseased with laurel wilt (Mayfield et al. 2008). In growth chamber experiments, some avocado plants wilted after artificial inoculation with the laurel wilt pathogen (Fraedrich et al. 2008). Furthermore, avocado wood has been demonstrated to be attractive to *X. glabratus* in field trapping trials (Hanula et al. in press). Concern exists that laurel wilt and *X. glabratus* could negatively impact the commercial avocado industry in south Florida and beyond.

There are 3 distinct races of avocado, West Indian (WI) (*Persea americana* var. *americana* Mill), Guatemalan (G) (*P. americana* var. *guatemalensis* Williams) and Mexican (M) [*P. americana* var *drymifloia* (Schlect. and Cham.)]. In Florida, West Indian and West Indian-Guatemalan hybrids are commercially grown; in California, mostly Guatemalan-Mexican hybrids are grown.

Two no-choice experiments were conducted to determine (1) whether *Xyleborus glabratus* would bore into healthy young avocado plants of various genetic backgrounds, and (2) whether these beetles could transmit the laurel wilt pathogen (*Raffaelea* sp.) into those plants. Stem sections (approx. 30 cm long and 10 cm in diameter) from wilted redbay trees infested with *X. glabratus* were collected from Jennings State Forest, Clay County, FL in early May 2007 and placed in rearing containers at the Florida DACS Division of Plant Industry in Gainesville, FL. Emerging adult females were collected, sealed in Petri plates and transferred to a quarantine greenhouse at the University of Florida, Indian River Research & Education Center in Fort Pierce, FL. Experimental plants were obtained in 3.8-L pots from a commercial nursery near Homestead, FL and ranged from 1.2-4.4 cm in basal diameter and 43.0-94.0 cm in height. Experiment 1 included 4 plants from each of 4 avocado cultivars representing different genetic backgrounds: 'Hass' (G-M hybrid), 'Simmonds' (WI), 'Monroe' (G-WI hybrid), and 'Winter Mexican' (M). These particular cultivars were of interest because 'Hass' is a widely grown commercial cultivar in California, 'Simmonds' and 'Monroe' are popular Florida cultivars, and 'Winter Mexican' is a pure Mexican race cultivar. Within each cultivar, 2 plants were ran-

domly assigned to receive a beetle release treatment and 2 plants served as untreated controls. In mid-Jun, 4 live beetles were released into a fine mesh sleeve that enclosed the main stem of each release-treatment plant from just above the soil to a height of 25-30 cm. Experiment 2 included 4 redbay, 4 avocado [cultivar 'Catalina' (G-WI)] and 3 live oak (*Quercus virginiana* Mill.) plants. Redbay and live oak were included as a known, highly-susceptible host species and a suspected non-host species, respectively. On 21 Aug 2007, 5 beetles per plant were released onto 2 plants of each of these species as described for experiment 1 and the remaining plants served as controls. Mesh sleeves were attached to control as well as release-treatment plants in experiment 2. In both experiments, plants were maintained in a greenhouse under natural sunlight periods and were watered every 1-2 d as needed. Typical greenhouse temperatures ranged from 23-34°C and relative humidity ranged from 37-82%.

Plants were monitored for evidence of beetle entry (including entrance holes and boring dust) and wilt symptoms. Plants that completely wilted and died before the end of the experiments were harvested and stem pieces were placed in plastic Ziploc® bags in a freezer. On 5 Nov 2007, all plants from both experiments were harvested, debarked along the main stem, and examined for dark outer sapwood discoloration. Wood chips from the main stem of all plants were surface sterilized by submersion in 5% sodium-hypochlorite for 30 s, rinsed in sterile water, and plated on cycloheximide streptomycin malt agar (CSMA), a medium selective for *Raffaelea* sp. and related anamorphs of the genus *Ophiostoma* (Harrington 1981). Plates were incubated for 2 weeks at room temperature and evaluated for presence of *Raffaelea* sp.

In both experiments, *X. glabratus* bored into the 2 release-treatment plants of redbay and all the avocado cultivars within 1-5 d of release, but did not bore into live oak (Table 1). Redbay plants attacked by *X. glabratus* wilted within 2-3 weeks after beetle entry and died within 4-10 weeks. The 'Simmonds' (WI) avocado plants attacked by *X. glabratus* began to wilt within 2-4 weeks after beetle entry and completely died within 11 weeks. One 'Monroe' (G-WI) plant wilted in less than 1/3 of its crown about 3 weeks after beetle entry, but after abscising the wilted leaves the plant recovered and did not exhibit any additional external symptoms. All other release-treatment and control plants remained externally asymptomatic through the end of the experiments. The *Raffaelea* sp. was isolated from discolored sapwood in the release-treated plants of redbay and all avocado cultivars except 'Hass' (G-M) (Table 1). In both experiments, sapwood discoloration was not observed in control plants of any cultivar or species and *Raffaelea* sp. was not isolated.

These experiments demonstrate that *X. glabratus* will readily bore into healthy young avocado plants of various genetic backgrounds and redbay plants when given no other choice, and can transmit the laurel wilt pathogen (*Raffaelea* sp.) into the xylem of these species. Dark outer sapwood discoloration, observed only in plants into which *X. glabratus* bored and from which the *Raffaelea* sp. was also obtained, is a characteristic host response in trees with laurel wilt disease (Fraedrich et al. 2008; Mayfield et al. 2008). As expected, outer sapwood discoloration was not observed in control plants nor in un-attacked plants of the suspected non-host, live oak. Why the *Raffaelea* sp. was not obtained nor sapwood discoloration observed in attacked plants of the 'Hass' cultivar is uncertain, but it may suggest that the genetic background of some avocado cultivars imparts some level of resistance to the pathogen and/or the development of the disease. The possibility of variable levels of resistance among avocado plants or cultivars is also consistent with the

TABLE 1. RESULTS OF RELEASING FEMALE *XYLEBORUS GLABRATUS* ONTO 2 PLANTS OF VARIOUS *PERSEA AMERICANA* CULTIVARS, *P. BORBONIA*, AND *QUERCUS VIRGINIANA*.

Exper.[a]	Host species	*Xyleborus glabratus* bored in[b]	Plants wilted & died[c]	Discolored sapwood[d]	*Raffaelea* sp. isolated[d]
1	*Persea americana* cv. Hass	x			
1	*P. americana* cv. Simmonds	x	x	x	x
1	*P. americana* cv. Monroe	x		x	x
1	*P. americana* cv. Winter Mexican	x		x	x
2	*P. americana* cv. Catalina	x		x	x
2	*P. borbonia*	x	x	x	x
2	*Quercus virginiana*				

[a]Four to 5 beetles per plant released into mesh sleeves on treatment plants in mid Jun 2007 (Experiment 1) and on 21 Aug 2007 (Experiment 2).
[b]*X. glabratus* bored into treated plants within 1-5 d of release.
[c]Plants wilted and died within 4-11 weeks after beetle entry.
[d]Plants harvested, debarked, evaluated for outer sapwood discoloration, and wood plated on selective media on Nov 5, 2007.

observations that some infected plants remained externally asymptomatic ('Winter Mexican' and 'Catalina') while others wilted and died ('Simmonds') and still another wilted but recovered (one 'Simmonds' plant). Similar variability in external symptoms was observed by Fraedrich et al. (2008) among 20 potted avocado plants (of unknown genetic background) artificially inoculated with the *Raffaelea* sp. Although disease development appeared to vary among cultivars of different genetic background in these experiments, testing of larger plants under field conditions with additional replication would be necessary before conclusions about the relative susceptibility of specific cultivars to laurel wilt disease can be made. The potential for young avocado plants to be attacked and infected as demonstrated in this study, the devastating effects of laurel wilt on closely-related redbay (Fraedrich et al. 2008), and the field-documented occurrence of the disease in a mature avocado (Mayfield et al. 2008) warrant additional research on the susceptibility of various avocado cultivars and races to the laurel wilt pathogen and its vector.

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Summary

In 2 no-choice experiments, female redbay ambrosia beetles were released onto young plants of redbay, live oak, and 5 different avocado cultivars of various genetic backgrounds. Beetles bored into all species except live oak, and transmitted the laurel wilt fungus to redbay and all the avocado cultivars except 'Hass'. Only redbay and 'Simmonds' avocado plants were killed, suggesting that avocado susceptibility to laurel wilt may vary among plants or cultivars of different genetic background.

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