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Source: Florida Entomologist, 95(3): 783-787

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.095.0334

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SUSCEPTIBILITY OF *PERSEA* SPP. AND OTHER LAURACEAE TO ATTACK BY REDBAY AMBROSIA BEETLE, *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

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Redbay ambrosia beetle (RAB), Xyleborus glabratus Eichhoff (Coleoptera: Curculionidae: Scolytinae), a native of Asia, was first discovered in the USA near Savannah, Georgia in 2002 (Haack 2001; Rabaglia et al. 2006). RAB is an effective vector of Raffaelea lauricola T.C. Harr., Fraedrich & Aghayeva (Harrington et al. 2008) that causes laurel wilt (LW), a lethal disease of several trees in the Lauraceae in the southeastern USA (Crane et al. 2008; Mayfield et al. 2008). Ambrosia beetle adults bore through the bark and into the xylem (wood) where they lay eggs, then adults and larvae cultivate and feed on symbiotic ambrosia fungi that grow in the galleries. Native Persea (Laurales: Lauraceae) species appear to be preferred hosts. LW is responsible for high mortality of redbay [P. borbonia (L.) Spreng.], swampbay [P. palustris (Raf.) Sarg.], and sassafras [Sassafras albidum (Nuttall) Nees] in Alabama, Florida, Georgia, Mississippi, North Carolina and South Carolina (Fraedrich et al. 2008; Hanula et al. 2008; Gramling 2010). As LW encroaches upon the Lake Wales Ridge ecosystem in southcentral Florida, silkbay (*P. humilis* Nash) is also showing susceptibility to LW and is dying. Additional species affected by LW include avocado (P. americana Mill.), spicebush [Lindera benzoin (L.) Blume], and other woody Lauraceae (Fraedrich et al. 2008) (Table 1).

The susceptibility of 5 avocado cultivars of Mexican, Guatemalan or West Indian origin to RAB and LW was demonstrated by Mayfield et al. (2008). However, with more than 23 West Indian cultivars grown in Florida, it is necessary to determine their susceptibility. Moreover, as an adventive species to the North American continent, RAB might affect other valuable New World species. Most *Persea* species are of Mexican, Central American, or South American origin. These *Persea* may have significant value in germplasm

collections, some have been discovered recently, and some have resistance to diseases that afflict their commercial relative, the avocado (Skutch et al. 1992; Scora & Bergh 1992; Zentmyer & Schieber 1992). Thus, their susceptibility to RAB and LW warrants evaluation. Another member of the Lauraceae of much concern is the California bay laurel [Umbellularia californica (Hook. & Arn.) Nutt.], a dominant hardwood species of the U.S. Pacific Coast. Through inoculation experiments, Fraedrich (2008) demonstrated that U. californica is susceptible to LW. With continued westward spread of LW, the host status of U. californica needs to be confirmed.

The 3 studies presented here evaluate susceptibility to RAB and LW in: 1) 13 West Indian avocado cultivars; 2) 10 non-commercial Persea spp., 1 Beilschmidia sp. (a genus related to Persea), and 3) U. californica. First, no-choice experiments were conducted to determine if RAB would bore into avocado cultivars not screened previously by Mayfield et al. (2008) and following similar methodology. Avocado cultivars 'Bernecker', 'Beta', 'Brooks late', 'Choquette', 'Donnie', 'Dupuis', 'Hall', 'Loretta', 'Lula', 'Monroe', 'Simmonds', 'Tower 2' and 'Waldin' (4 plants each) were planted in 10-gallon pots in a screenhouse at the Plant Sciences Research and Education Unit, University of Florida (UF), Citra, Florida in VI-2008. Two plants of each cultivar were infested by enclosing 4 newly emerged ♀ RAB (UF colonyreared) within a mesh sleeve on the lower trunk. Two plants per cultivar were uninfested controls. Entrance holes and perseitol (white exudate from wounds) were monitored for 4 wk. Severity of wilt symptoms was scored using the following LW index: 0 = no wilt; 1 = wilt, no leaf necrosis; 2 = wilt,10% necrosis or defoliation; 3 = wilt, 30% necrosis/ defoliation; 4 = 50% necrosis/defoliation; 5 = 75%necrosis/defoliation; 6 = 100% necrosis/defoliation

Table 1. List of reported and potential hosts of Xyleborus glabratus.

Species name	Provenance	Evidence of Reproduction by RAB	Evidence of Evidence of Reproduction infection by BAB R. lauricola	Evidence of boring by RAB	of Reference
Beilschmidia sp.				*	reported here
Cinnamomum camphora (L.) J. Presl.	Taiwan, China, Japan		*		Smith et al .2009
Cinnamomum jensenianum HandMazz.	China		*		Fraedrich (ppt)
Laurus noblis L.	Southern Europe		*		Fraedrich (ppt)
Lindera benzoin (L.) Blume	Eastern USA		*	*	Fraedrich et al. 2008
Lindera latifolia Hk.f					Wood and Bright 1992
Lindera melissifolia (Walter) Blume			*		Fraedrich et al. 2008
Lindera strychnifolia (Sims) Kosterm	China, Japan		*		Fraedrich (ppt)
Litsea aestivalis		*	*	*	-Hughes et al. 2011
Litsea elongata (Nees) Hk.f					Wood and Bright 1992
Machilus thunbergii Siebold & Zucc.	Taiwan, China, Japan		*		Fraedrich (ppt)
Ocotea coriacea (Sw.) Britton			*		Fraedrich (ppt)
Persea americana Mills. cv. 'Hass'	Mexico, Central America		*	*	Mayfield et al. 2008
P. americana Mills. cv. 'Simmonds'			*	*	Mayfield et al. 2008
P. americana Mills. cv. 'Monroe'			*	*	Mayfield et al. 2008
P. americana Mills. cv. 'Winter Mexican'			*	*	Mayfield et al. 2008
P. americana Mills. cv. 'Catalina'			*	*	Mayfield et al. 2008
P. americanaMills. cv. 'Bernecker'				*	reported here
P. americanaMills. cv. 'Beta'				*	reported here
P. americanaMills. cv. 'Brookslate'				*	reported here
P. americanaMills. cv. 'Choquette'				*	reported here
P. americanaMills. cv. 'Donnie'				*	reported here
P. americanaMills. cv. 'Dupuis'				*	reported here
P. americanaMills. cv. 'Hall'				*	reported here
P. americanaMills. cv. 'Loretta'				*	reported here
P. americanaMills. cv. 'Lula'				*	reported here
P. americanalMills. cv. 'Tower 2'				*	reported here
P. americanalMills. cv. 'Waldin'				*	reported here
Persea borbonia (L.) Spreng.	Southern USA	*	*	*	Fraedrich et al. 2008, Hanula et al. 2008
Persea humilis Nash	Southern USA, only Florida?		*	*	Fraedrich et al. 2008, Hanula et al. 2009
Persea palustris (Raf.) Sarg.	Eastern USA	*	*	*	Fraedrich et al. 2008
Persea caerula (Ruiz and Pav.) Mez	Peru, Amazon	*		*	reported here
Persea pachypoda Ehrenb	Mexico, California, South America	*		*	reported here
Persea floccosa Mez.,			*	*	reported here

Table 1. (Continued) List of reported and potential hosts of $X_{YLEBORUS}$ glabratus

of y Reference	reported here reported here	reported here	reported here	reported here	reported here	Fraedrich (ppt)	Fraedrich et al. 2008	Fraedrich 2008; ?reported here
Evidence boring b RAB	* *	*	*	*	*		*	*
Evidence of Evidence of Evidence of Reproduction infection by boring by BAB R. lauricola RAB						*	*	*
Evidence of Reproduction by RAB	*				*		*	
Provenance						Mexico, Guatemala	Central and Eastern USA	
Species name	Persea skutchii L. O. Williams Persea nubigiena L. O. Williams	Persea indica Zentmyer & Schrieber	Persea tolimanensis Zentmyer & Schrieber	Persea cinerascens	Persea tilarensis	$Persea\ liebmanni\ (=P.\ podedemia)$	Sassafras albidum (Nuttall) Nees	Umbellularia californica

(Peña et al. 2011). After 2 wk, all cultivars had 1-2 entrance holes (df_{8,36}; F = 2.18; Pr > F = 0.052) (Table 2). This is an important indication of successful beetle boring. The LW index assessed during the last wk of the experiment fluctuated between 0 - 1.8 (df $_{8.38}$; F = 1.85; Pr > F = 0.10), which corresponded to an average between wilt only and 10% leaf necrosis (Table 2). On 22-VIII-2008 all plants were harvested, and wood chips were collected, surface sterilized, and plated on medium selective for Raffaelea lauricola (Mayfield et al. 2008). Isolation frequency ranged from 0-50% for the different cultivars (Table 2). These results are in in agreement with the results of Mayfield et al (2008) who reported that when given no choice, RAB can bore into and transmit the pathogen R. lauricola into the xylem of avocado, which characteristically presents as dark discoloration of the outer sapwood

In the second study, RAB attraction to 10 Persea spp. and 1 Beilschmidia sp. was tested in the field and laboratory. Four replicate logs (33 cm $long \times \sim 2.5$ cm diam.) of P. caerulea Ruiz and Pav., P. borbonia, P. pachypoda Ehrenb [syn: Cinnamomum pachypodum (Nees) Kosterm., Phoebe pachypoda (Nees) Mez], P. floccosa Mez., P. skutchii C. K. Allen, P. nubigiena L. O. Williams, P. indica (L.) Spreng., P. tolimanensis Zentmyer & Schrieber (also called "aguacate de mico", a Central American species), P. cinerascens S. F. Blake, P. tilarensis and Beilschmidia were hung in full sun ~1.2 m high near infested redbay trees with an approx. distance of 10 m between adjacent treatments in Hastings, Florida from IX to X-2009. After 30 d, the logs were collected and the number of entry holes determined under a stereomicroscope. Logs were placed individually in cardboard containers for beetle emergence at 26 °C and 70-80% RH for 60 d. Bolts of P. skutchii, P. cinerascens and P. indica appeared to be preferred by ambrosia beetles over other *Persea* spp., including *P. borbonia* (Table 3). Unfortunately, no beetle emerged from these bolts, perhaps due to desiccation of bolts under field or storage conditions.

Another survey was set up at Hickory Hammock, a 4,000-acre (1,619 ha) natural preserve in Highlands County, Florida (27°25'35"N, 81°9'42" W). This site was known since 2009 to have LW and RAB. Bolts (same species as above) were hung on 23-II-2010 on the sunny edge of a trail, removed 30 d later, and stored as described above. With the exception of *P. floccosa*, bolts of all species had entrance holes (df_{10,10}; F = 2.04; $Pr > \hat{F} =$ 0.13). RAB emerged from P. caerula and P. tilarensi; Ambrosiodmus lecontei Hopkins (Curculionidae: Scolytinae) from P. nubigena, P. pachypoda and P. tilarensis; and Xylosandrus crassiusculus $(Motschulsky)\ (Curculionidae:\ Scolytinae)\ (df_{_{10,10}};$ F = 1.61; Pr > F = 0.23) (Table 3). No beetle emerged from other Persea spp. However, lack

Table 2. Mean infestation by Xyleborus Glabratus and severity of laurel wilt observed after 4 wk in no-choice tests with 13 West Indian Avocado cultivars, young potted trees (2 per cultivar) were exposed to 4 X. Glabratus females enclosed in a sleeve at the base of the trunk.

Avocado Cultivar	Number of holes/plant ± SE	Holes with Perseitol/plant ± SE	LW Severity Index	R. lauricola isolation frequency
Bernecker	1.50 ± 0.50	1.50 ± 0.00	1.50 ± 0.27	0.25
Beta	2.00 ± 0.00	2.00 ± 0.00	0.40 ± 0.16	0.50
Brookslate	1.50 ± 0.50	1.50 ± 0.50	0.00 ± 0.00	0.00
Choquette	2.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00	0.25
Donnie	2.50 ± 0.50	2.50 ± 0.50	1.00 ± 0.33	0.50
Dupuis	1.50 ± 0.50	1.50 ± 0.50	0.00 ± 0.00	0.00
Hall	2.00 ± 0.00	2.00 ± 0.00	1.30 ± 0.26	0.50
Loretta	1.00 ± 1.00	1.00 ± 1.00	0.00 ± 0.00	0.00
Lula	2.00 ± 0.00	2.00 ± 0.00	0.70 ± 0.00	0.25
Monroe	2.00 ± 0.00	2.00 ± 0.00	0.40 ± 0.16	0.25
Simmonds	1.50 ± 0.50	1.50 ± 0.50	1.70 ± 0.57	0.50
Tower 2	2.00 ± 1.00	2.00 ± 1.00	1.50 ± 0.17	0.00
Waldin	2.00 ± 0.00	2.00 ± 0.00	1.80 ± 0.13	0.50

Numbers followed by a different letter were significantly different at $\alpha = 0.05$; GLM procedure; Tukey's Studentized Range (HSD) test; SAS, 2008.

of emergence from those species could be due to desiccation and not the result of plant resistance.

On 24-II-2010, host boring bioassays were set up in the laboratory, using methods similar to Kendra et al. (2011). Bolts (10.9 \times 2.7 cm diam) of the same species above (2 replicates per species) were cut and immediately placed individually in glass jars (0.95 liter) with 200 mL of water to prevent desiccation. Five $\mbox{$^{\circ}$}$ newly emerged RAB were placed on top of each bolt and kept for 24 h at 22 \pm 2 °C and 12:12 h L:D. RAB boring was recorded at 1, 2, 3, 4 and 24 h. RAB bored into all species except *P. floccosa*, and infestation varied from 1.5 to 4 beetles boring per bolt (Table 3) (

df, $_{10,11}$; F = 3.79, Pr > F = 0.02) (GLM procedure, Tukey's Studentized Range (HSD) Test (SAS, 2008). P. floccosa is a Guatemalan-type species, which is believed to be the most ancient form of Persea (Scora & Bergh 1992).

In the third experiment, bolts of redbay, avocado and California bay laurel were hung at Ordway-Swisher Biological Station, University of Florida, Gainesville (N 29° 41.040, W 082° 22.109). Nine logs of each species were hung in an area where both diseased and healthy red bay were present, and left in the field for 1 mo (18 IX-19-X-2009). Logs were brought into the laboratory, bore holes were measured, and those of appropriate diam-

Table 3. Susceptibility of *persea* species to attack by *Xyleborus glabratus* and other scolytinae in Florida. Bolts were hung for 30 days in field tests. Bolts were exposed to 5 female *X. glabratus* in no-choice laboratory bioassays.

	Field Test 1	Field Test 2		Laboratory Test
Plant Species Tested	$\begin{array}{c} {\rm Entry} \\ {\rm Holes/Bolt \pm SE} \end{array}$	Entry Holes/Bolt \pm SE ¹	Emerging Beetles/Bolt ± SE	Number of X . $glabratus$ entrances/bolt $\pm SE^1$
P. caerulea P. borbonia	2.81 ± 2.57 0.37 ± 0.14	0.50 ± 0.50 1.00 ± 1.00	0.50 ± 0.50 0.00 ± 0.00	3.00 ± 0.00 a 3.50 ± 0.50 a
P. pachypoda	1.73 ± 1.00	3.50 ± 1.50	1.00 ± 1.00	$3.00 \pm 1.00 a$
P. floccosa	0.76 ± 0.28	0.00 ± 0.00	0.00 ± 0.00	$0.00 \pm 0.00 \text{ b}$
P. skutchii	5.61 ± 2.28	1.50 ± 1.50	0.00 ± 0.00	2.50 ± 0.50 a
P. nubigiena	2.14 ± 2.11	7.00 ± 3.00	2.50 ± 2.50	2.50 ± 0.50 a
P. indica	4.11 ± 2.71	0.50 ± 0.50	0.00 ± 0.00	1.50 ± 0.50 a
P. tolimanensis	1.57 ± 1.13	2.00 ± 2.00	0.00 ± 0.00	2.50 ± 0.50 a
Beilschmidia sp.	2.14 ± 1.95	0.50 ± 0.50	0.00 ± 0.00	$3.00 \pm 1.00 \text{ a}$
P. cinerascens	8.49 ± 6.71	2.00 ± 1.00	0.00 ± 0.00	$4.00 \pm 0.00 \text{ a}$
P. tilarensis	1.81 ± 1.57	4.00 ± 4.00	4.00 ± 4.00	3.50 ± 0.50 a

¹Means followed by a different letter are significantly different (GLM Procedure; Tukey Studentized Range (HSD) Test; SAS 2008)

eter for RAB (0.8 mm, Hanula et al. 2008) were counted and recorded. No dissection of the galleries was made. Surprisingly, no RAB entry holes were found on redbay logs, while entrance holes were recorded on avocado (0.55 \pm 0.29) and California bay laurel (0.22 \pm 0.14). However, no beetle emerged from these bolts. Field tests and lab bioassays conducted during 2011 have shown that female RAB are highly attracted to, and will bore into freshly-cut bolts of *U. californica* (P. E. Kendra, unpubl.; A. E. Mayfield, unpubl.).

Summary

These preliminary results indicate that there are numerous New World species of the Lauraceae potentially at risk of attack by *X. glabratus*. More research is needed to fully determine the susceptibility of *Persea* spp. and other genera within the Lauraceae to both the pathogen and vector. However, because of the difficulty in obtaining bolts of non-native *Persea* species and other genera, efforts should be directed particularly at those species where an indication of non-susceptibility to *X. glabratus* has been observed.

ACKNOWLEDGMENTS

We thank Drs. R. Giblin-Davis and R. E. Litz for suggestions to improve the manuscript. We thank Jose Alegria, Ana Vargas and the personnel of the Plant Science Research Station of the University of Florida, IFAS, Citra, Florida for their help. This research was partially funded by the Florida Avocado Committee.

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