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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 south-central 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 colony-reared) within a mesh sleeve on the lower trunk. Two plants per cultivar were uninfested controls. Entrance holes and perithecia (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 infection by <i>R. lauricola</i>	Evidence of boring by RAB	Reference
<i>Beilschmidia</i> sp.				*	reported here
<i>Cinnamomum camphora</i> (L.) J. Presl.	Taiwan, China, Japan		*		Smith et al. 2009
<i>Cinnamomum jensenianum</i> Hand.-Mazz.	China		*		Fraedrich (ppt)
<i>Laurus nobilis</i> L.	Southern Europe		*		Fraedrich (ppt)
<i>Lindera benzoin</i> (L.) Blume	Eastern USA		*	*	Fraedrich et al. 2008
<i>Lindera latifolia</i> Hk.f					Wood and Bright 1992
<i>Lindera melissifolia</i> (Walter) Blume			*		Fraedrich et al. 2008
<i>Lindera strychnifolia</i> (Sims) Kosterm	China, Japan		*		Fraedrich (ppt)
<i>Litsea aestivalis</i>		*	*	*	-Hughes et al. 2011
<i>Litsea elongata</i> (Nees) Hk.f					Wood and Bright 1992
<i>Machilus thunbergii</i> Siebold & Zucc.	Taiwan, China, Japan		*		Fraedrich (ppt)
<i>Ocotea coriacea</i> (Sw.) Britton			*		Fraedrich (ppt)
<i>Persea americana</i> Mills. cv. 'Hass'	Mexico, Central America		*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Simmonds'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Monroe'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Winter Mexican'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Catalina'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Bernecker'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Beta'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Brookslate'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Choquette'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Donnie'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Dupuis'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Hall'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Loretta'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Lula'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Tower 2'			*	*	Mayfield et al. 2008
<i>P. americana</i> Mills. cv. 'Waldin'			*	*	Mayfield et al. 2008
<i>Persea borbonia</i> (L.) Spreng.	Southern USA	*	*	*	Fraedrich et al. 2008, Hanula et al. 2008
<i>Persea humilis</i> Nash	Southern USA, only Florida?		*	*	Fraedrich et al. 2008, Hanula et al. 2009
<i>Persea palustris</i> (Raf.) Sarg.	Eastern USA	*	*	*	Fraedrich et al. 2008
<i>Persea caerulea</i> (Ruiz and Pav.) Mez	Peru, Amazon	*	*	*	reported here
<i>Persea pachypoda</i> Ehrenb	Mexico, California, South America	*	*	*	reported here
<i>Persea floccosa</i> Mez.,			*	*	reported here

TABLE 1. (CONTINUED) LIST OF REPORTED AND POTENTIAL HOSTS OF *XYLEBORUS GLABRATUS*.

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

(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 *Raffaella lauricola* (Mayfield et al. 2008). Isolation frequency ranged from 0-50% for the different cultivars (Table 2). These results are 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 ~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. nubigena* 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 ($df_{10,10}$; $F = 2.04$; $Pr > F = 0.13$). RAB emerged from *P. caerulea* and *P. tilarensis*; *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	<i>R. lauricola</i> 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 × 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 ♀ newly emerged RAB were placed on top of each bolt and kept for 24 h at 22 ± 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.

Plant Species Tested	Field Test 1	Field Test 2		Laboratory Test
	Entry Holes/Bolt ± SE	Entry Holes/Bolt ± SE ¹	Emerging Beetles/Bolt ± SE	Number of <i>X. glabratus</i> entrances/bolt ± SE ¹
<i>P. caerulea</i>	2.81 ± 2.57	0.50 ± 0.50	0.50 ± 0.50	3.00 ± 0.00 a
<i>P. borbonia</i>	0.37 ± 0.14	1.00 ± 1.00	0.00 ± 0.00	3.50 ± 0.50 a
<i>P. pachypoda</i>	1.73 ± 1.00	3.50 ± 1.50	1.00 ± 1.00	3.00 ± 1.00 a
<i>P. floccosa</i>	0.76 ± 0.28	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 b
<i>P. skutchii</i>	5.61 ± 2.28	1.50 ± 1.50	0.00 ± 0.00	2.50 ± 0.50 a
<i>P. nubigiena</i>	2.14 ± 2.11	7.00 ± 3.00	2.50 ± 2.50	2.50 ± 0.50 a
<i>P. indica</i>	4.11 ± 2.71	0.50 ± 0.50	0.00 ± 0.00	1.50 ± 0.50 a
<i>P. tolimanensis</i>	1.57 ± 1.13	2.00 ± 2.00	0.00 ± 0.00	2.50 ± 0.50 a
<i>Beilschmidia</i> sp.	2.14 ± 1.95	0.50 ± 0.50	0.00 ± 0.00	3.00 ± 1.00 a
<i>P. cinerascens</i>	8.49 ± 6.71	2.00 ± 1.00	0.00 ± 0.00	4.00 ± 0.00 a
<i>P. tilarensis</i>	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 ± 0.29) and California bay laurel (0.22 ± 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.

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