

Eucalyptol is an Attractant of the Redbay Ambrosia Beetle, *Xyleborus Glabratus*

Emily H. Kuhns · Xavier Martini · Yolani Tribuiani ·
Monique Coy · Christopher Gibbard · Jorge Peña ·
Jiri Hulcr · Lukasz L. Stelinski

Received: 9 January 2014 / Revised: 20 February 2014 / Accepted: 28 February 2014 / Published online: 22 April 2014
© Springer Science+Business Media New York 2014

Abstract The redbay ambrosia beetle, *Xyleborus glabratus*, is an invasive wood-boring beetle that has become established in the southeastern United States. The beetle transmits the causal pathogen of lethal laurel wilt to susceptible host trees, which include redbay, an important forest community species, and avocado, a valuable food crop. By examining odors of redbay wood, we developed an artificial lure that captured *X. glabratus* in redbay forests. Eucalyptol was a critical component of the blend for beetle attraction, and eucalyptol alone in large quantities attracted *X. glabratus*. Furthermore, eucalyptol stimulated boring by *X. glabratus* into paper arenas. The results suggest that eucalyptol contributes to host selection behavior of *X. glabratus* and may be useful for management of this pathogen vector.

Keywords Avocado · Laurel wilt · *Raffaelea lauricola* · Boring stimulant · Kairomone · Host acceptance · Scolytinae · Invasive beetle · Coleoptera · Cuculionidae

Introduction

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an Asian ambrosia

beetle that recently has invaded the southeastern United States (Fraedrich et al. 2008; Harrington et al. 2011; Rabaglia et al. 2006). Once established in Georgia, this wood-boring pest spread rapidly, carrying with it a symbiotic fungus, *Raffaelea lauricola*, T.C. Harr. Fraedrich & Aghayeva that causes laurel wilt (Harrington et al. 2008). Unlike most other ambrosia beetle species, *X. glabratus* attacks healthy trees, inoculating them with *R. lauricola*, which causes wilting, and dieback of branches (Fraedrich et al. 2008). In susceptible trees, death of entire trees can follow within months of initial infection (Fraedrich et al. 2008, 2011; Mayfield et al. 2008a).

In the southeastern US, wild populations of Lauraceae, such as redbay, *Persea borbonia* (L.) Spreng., and swampbay, *Persea palustris* (Raf.) Sarg., have been decimated by laurel wilt (Formby et al. 2012; Fraedrich et al. 2007; Hanula et al. 2008). The beetles have spread throughout the avocado-growing region of South Florida (Miami-Dade County) in fragmented swampbay habitats (Laurel Wilt Distribution, 2013). Because avocado, *Persea americana* Mill., also is vulnerable to laurel wilt (Mayfield et al. 2008b), substantial negative impacts on the avocado industry are anticipated (Evans et al. 2010). To date, approximately 1800 avocado trees have been removed because of laurel wilt; however, it is unclear if the disease caused the initial decline or if the wilt occurred as a secondary disease (J.E. Peña, unpublished).

The threat to avocado has greatly increased research interest in this invasive pest. There is mixed evidence that avocado can be as attractive to *X. glabratus* as redbay odors (Hanula et al. 2008; Mayfield and Hanula, 2012). Odors from these species and several potential host and non-host trees, have been evaluated by gas chromatography/mass spectrometry (GC/MS; Niogret et al. 2011). Based on a consensus of sesquiterpene content among host species, α -copaene, β -caryophyllene, α -humulene, calamemnene, and cadinene were suspected to be responsible for attraction of *X. glabratus*. More specifically, α -copaene was hypothesized

E. H. Kuhns · X. Martini · Y. Tribuiani · M. Coy · C. Gibbard ·
L. L. Stelinski (✉)

Citrus Research and Education Center, Department of Entomology
and Nematology, University of Florida, Lake Alfred, FL 33850, USA
e-mail: stelinski@ufl.edu

J. Peña

Tropical Research and Education Center, Department of Entomology
and Nematology, University of Florida, Homestead, FL 33031, USA

J. Hulcr

School of Forest Resources and Conservation, University of Florida,
Gainesville, FL 32611, USA

to be a long-range attractant of *X. glabratus*, due its high abundance in the analyzed host-tree species (Hanula and Sullivan 2008; Niogret et al. 2011). Several botanical oil lures containing α -copaene have been evaluated as attractants for *X. glabratus*. Phoebe and manuka botanical oils have had varying success as lures for *X. glabratus* (Hanula and Sullivan 2008; Kendra et al. 2012). Phoebe oil is obtained from the leaves of *Phoebe porosa* Menz., while manuka oil is obtained from the leaves of *Leptospermum scoparium* J.R. Forst. & G. Forst. Cubeb oil, obtained from leaves of *Piper cubeba* Linn., also has been evaluated and is more effective as a lure for *X. glabratus* than is manuka oil alone (Hanula et al. 2013).

Recently, it is has been shown that avocado is a suboptimal host for *X. glabratus*, compared to redbay or swampbay (Brar et al. 2013; Carrillo et al. 2012; Mayfield and Hanula 2012; Peña et al. 2012). While it is known that *X. glabratus* females will bore into avocado trees (Kendra et al. 2011; Mayfield et al. 2008a), fewer beetles bore into avocado bolts than into redbay bolts (Hanula et al. 2008). More *X. glabratus* emerged from swampbay bolts than avocado bolts that were naturally infested with ambrosia beetles (Carrillo et al. 2012), and more *X. glabratus* adults emerged from swampbay and redbay than from avocado per area of tree (Brar et al. 2013). Also, adult *X. glabratus* emerging from redbay and swampbay are larger (measured by head capsule size) than those emerging from avocado (Brar et al. 2013). Based on these findings, there appears to be a fitness cost associated with *X. glabratus* development in avocado, compared to that in redbay or swampbay.

Differences in phytochemical content among host species might explain the variation in performance of *X. glabratus* among hosts. For example, once a host tree is located, the chemistry of the wood surface may affect the propensity of the beetle to bore. The role of host chemistry in boring stimulation has been shown in bark beetles (Walter et al. 2010), but has not been tested in ambrosia beetles, even though ethanol is a likely boring stimulant (Elliott et al. 1983). Taxonomically, ambrosia beetles are a subset of bark beetles; therefore, it is reasonable to assume that host chemistry could also affect boring behavior in ambrosia beetles. As *X. glabratus* bores into the xylem of a tree, the wood is inoculated with a multitude of microorganisms associated with the beetle, including the symbiotic fungus *R. lauricola*, on which the beetle and larvae feed. The poorer performance observed on avocado, as compared to swampbay or redbay, may be associated with establishment and growth of *R. lauricola* within avocado trees. The phytochemistry of wood may affect the complex microbial community within galleries, thus altering the quantity or quality of *R. lauricola* available to nourish the beetle and offspring. Therefore, differences in host phytochemistry between avocado and redbay may be involved in the observed differences in host suitability (Brar et al. 2013).

In this study, we examined two stages of host selection by *X. glabratus*: 1) long-range attraction to host odors, and 2) boring behavior upon a substrate in the presence of host odors. We initiated our study with a comparison of odors from a preferred host (redbay) versus a suboptimal one (avocado). We hypothesized that chemical constituents that were abundant in redbay, but absent or less abundant in avocado, may be responsible for the difference in host acceptance by *X. glabratus* between redbay and avocado. Identification of chemicals unique to redbay trees and that are attractants of *X. glabratus* may result in development of potent lures for use in commercial avocado settings, where there would presumably be no competition between lure and host-plant volatiles.

Methods and Materials

Chemicals Eucalyptol (C80601), *p*-cymene (C121452), myrcene (M100005), limonene (62122), sabinene (W530597), α -pinene (147524), and β -pinene (W290300) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Manuka lures (#3083) were purchased from Synergy Semiochemicals Corp. (Burnaby, BC Canada), and consisted of an internal matrix soaked in manuka oil, housed within a plastic pouch. Prototype eucalyptol lures were made by Alpha Scents (West Linn, OR, USA) and consisted of a 10 × 15 cm polyethylene bag containing a foam matrix and eucalyptol.

Volatile Collection and Analysis Avocado wood samples were taken from mature avocado groves in Homestead, FL, USA. Redbay samples were taken from mature redbay trees from Lake Kissimmee State Park, FL, USA under permits 08181113 and 12071213 issued by Florida Department of Environmental Protection. All trees appeared free of the pathogen at the time of sampling. Avocado samples were taken from three representative cultivars (Booth, Lula, and Peterson). Approximately 6–10 cm diam. branches were cut into 3–5 cm thick discs and the cut wood placed in clean 250 ml glass beakers, which were then covered tightly with aluminum foil. The headspace volatiles of the wood were allowed to equilibrate for at least 15 min at 21 °C. A triphase 50/30 μ m DVB/Carboxen/PDMS StableFlex™ solid phase micro-extraction (SPME; Sigma Aldrich) fiber was punctured through the tin foil lid and the fiber exposed for 15 min. The SPME was desorbed, splitlessly, for 5 min. at 240 °C. Helium was used as a carrier gas at 2 ml.min⁻¹, and the odor constituents chromatographed on a Stabilwax capillary column (60 m × 0.25 mm diam; 0.5 μ m film thickness; Restek, Bellefonte, PA, USA) using a temperature program of 40–240 °C at 7 °C.min⁻¹. Identification of the compounds was performed with a Perkin Elmer Clarus 500 quadrupole mass spectrometer and Turbo Mass software (Waltham, MA, USA). Retention times of authentic standards, when available, and

matching mass spectra to the NIST database were used to identify components.

Field Trials of Lures The research site was located within Wekiwa Springs State Park, a protected ecosystem, and the research was conducted under permits 08181113 and 12071213 issued by Florida Department of Environmental Protection. No protected species were sampled during experiments. The trapping location is described in greater detail in Kuhns et al. (2014). White-colored elm beetle sticky traps (IPM-BBT-12, Great Lakes IPM, Inc., Vestaburg, MI, USA) were cut in half resulting in a 46 × 32 cm sticky panel. Each panel was stapled, 1 m above ground level, to a wooden stake, and placed within five replicate blocks along a redbay habitat. Within-block traps were no less than 6 m apart, and blocks were a minimum of 60 m apart. In the first experiment, sticky traps were baited with: negative control (unbaited), manuka lure (positive control), redbay full blend (RB Full;), redbay blend without eucalyptol (RB-eucalyptol), redbay blend without *p*-cymene (RB-Cymene), or redbay blend without α -pinene (RB- α -Pinene). The numbers of *X. glabratus* captured over a 2 week trapping period were determined. Six replicates of the lures were placed in blocks within a redbay-infested forest. The composition of each of these blends used in field trials is listed in Table 1 and described in the Results section. Five ml of each blend were pipetted into a 7 ml BEEM vial (Thermo Fisher Scientific, Waltham, MA, USA) and the vial was sealed with hot glue (#BSS6-4; Arrow Fastener Co., LLC, Saddle Brook, NJ, USA). A 1 mm hole was punctured in the lid of the vial, and the lure was then affixed to the sticky trap. Traps were monitored for 2 week from January 10–24, 2013.

In the second experiment, we evaluated a high release of a proprietary eucalyptol lure, the original RB Full lure alone, as well as positive (manuka lure) and negative (unbaited traps)

controls on sticky traps. The number of *X. glabratus* captured over a 2 week trapping period was determined. Six replicates of these lures were tested in blocks from March 14–28, 2013, at the same locations as the first experiment.

Release Rates of Lures Four manuka, four redbay full blend, and two eucalyptol lures were deployed outdoors from April 22–May 30, 2013 in Lake Alfred, FL. The lures were weighed periodically to calculate gravimetric release. The data were fitted with exponential decay curves, and the average release rates from days 1–4 and 8–9 were calculated.

Beetle Boring Assay Beetles were field collected from Wekiwa Springs State Park using a combination of panel and soda bottle-style (Ranger et al. 2010) live traps baited with manuka lures. The traps were modified to funnel captured beetles into a 50 ml conical tube that contained moistened laboratory tissue (Kimberly Clark, Roswell, GA, USA). Beetles were collected weekly and were sorted and used in laboratory bioassays within 48 h of collection.

Boring behavior was assayed with ten beetles using paper substrates treated with one of the four treatment types: 1) control, 2) RB Full, 3) RB-eucalyptol, or 4) eucalyptol alone. A 7.5 μ l aliquot of each blend was diluted in ethanol to a final volume of 1.5 ml. A 2.8 μ l aliquot of eucalyptol (the equivalent amount found in the full blend) was diluted in ethanol to a final volume of 1.5 ml. Ethanol alone was used for the control treatment. Chromatography paper strips (1 × 7 cm) were treated with 100 μ l of the diluted odor treatments, and the solvent was allowed to evaporate. The paper strips were tightly rolled, slid into 0.6 ml BEEM vials, and then moistened with 100 μ l of sterile water. The approximate dimensions of the paper boring substrate, once rolled, were 5 mm diam. × 10 mm length. A single live *X. glabratus* adult female was enclosed in each tube and allowed to bore into the paper over 4 d. The presence or absence of boring was recorded for each beetle daily, to determine the time required to initiate boring.

Statistical Analyses Analyses were performed with the statistical software R (v. 3.0.1; <http://www.r-project.org>). For the first experiment, we used a linear mixed-effect model (package nlme and function lme in R), with a Gaussian distribution to determine if presence or absence of manuka, α -pinene, *p*-cymene, or eucalyptol in lures had an effect on the numbers of beetles captured on sticky traps. The data were log transformed to normalize the distribution. The presence or absence of manuka, α -pinene, *p*-cymene, or eucalyptol in each trap was computed in a matrix with 0 for absence and 1 for presence. Presence and absence of these compounds were the fixed terms of the model, and trap location was the random term.

Table 1 Synthetic odor blends based on the chemistry of redbay wood¹

Compound	% volume			
	RB Full	RB-Eucalyptol	RB-Cymene	RB- α -Pinene
α -pinene	12.2	19.5	19.9	-
β -myrcene	2.7	4.3	4.4	3.1
β -pinene	4.4	7.0	7.2	5.0
cymene	38.5	61.5	-	43.9
eucalyptol	37.4	-	60.8	42.6
limonene	3.6	5.8	5.9	4.1
sabinene	1.2	1.9	1.9	1.3

¹ Four synthetic redbay odor blends were created using the percent abundance based on gas chromatography/mass spectrometry analyses. The synthetic blends were used in field trials of lures for attractiveness to *Xyleborus glabratus*. The Redbay Full and Redbay-eucalyptol blends were further tested in beetle boring assays

Regarding the second experiment, the variance differed significantly among treatments and the data were not normally distributed. Consequently, we performed a Generalized Linear Model (GLM) with a log link function for the Poisson distribution to determine if the number of *X. glabratus* captured in traps baited with manuka, RB Full, or eucalyptol alone was different among treatments. The data also showed overdispersion, and we consequently corrected the standard errors using a quasi-GLM model, in which the variance is given by $\varphi \times \mu$, where φ is the dispersion parameter and μ the mean (Zuur et al. 2009). We began with a model that included lure treatment and field location: *Number of Beetles Captured* ~ *Lure + Location*. The location factor was not significant ($P > 0.100$) and was removed from the model to obtain a minimal adequate model (Crawley 2009). A *post-hoc* Tukey test (function *glht* of the package *multcomp*) was performed to determine what treatments (lure combinations) were differentially attractive to beetles.

Finally, for the boring experiment, we measured the time to initiate boring for beetles that bored into paper arenas over the course of 4 d. We used Cox's proportional hazards model (Cox 1972; Crawley 2009) to determine if the time to initiate boring differed among treatments over the course of the experiment.

Results

Volatile Analysis of Persea Wood We examined the odors of redbay and avocado wood in order to identify compounds that may be attractive to *X. glabratus*. Representative mass chromatograms are shown in Fig. 1. Most notably, the wood of the avocado varieties released less eucalyptol and *p*-cymene than did redbay wood. Of the three avocado varieties, Peterson appeared most similar, qualitatively, to redbay (Fig. 1). Peterson avocado wood released monoterpenes (α -pinene, β -pinene, sabinene, myrcene, and limonene) that were also released by redbay; the other two avocado varieties did not release detectable levels of these monoterpenes, with the exception of low levels of α -pinene and limonene in Lula and Booth varieties, respectively. Qualitatively, major sesquiterpene constituents, including cubebene, α -copaene, and β -caryophyllene, were similar between redbay and the avocado varieties.

Field Trapping In the first field experiment, we evaluated the attractiveness of redbay odors (Fig. 2), specifically with respect to the presence or absence of the three major monoterpene/monoterpenoid components of the blend. We also compared these beetle captures with the captures in traps baited with manuka lure (positive control) or unbaited traps (negative control). We found that the absence of *p*-cymene

($P=0.848$) and α -pinene ($P=0.339$) in the RB blends had no effect on the number of *X. glabratus* captured as compared to negative controls (Table 2). However, presence of eucalyptol resulted in more *X. glabratus* captured, as compared with the control ($P < 0.001$, Table 2). Manuka-baited traps also caught more *X. glabratus* than the negative control ($P < 0.001$, Table 2).

In the second field experiment, we compared traps baited with manuka, eucalyptol alone, RB Full, and negative control traps (Fig. 3). We observed a difference in *X. glabratus* capture among treatments ($F_{3, 30}=9.698$, $P < 0.001$). Capture of *X. glabratus* in the high dosage eucalyptol-baited traps was equivalent to that observed in manuka-baited traps. While the RB Full blend-baited traps caught more beetles than the negative control traps, the difference was not significant ($P=0.058$; Fig. 3).

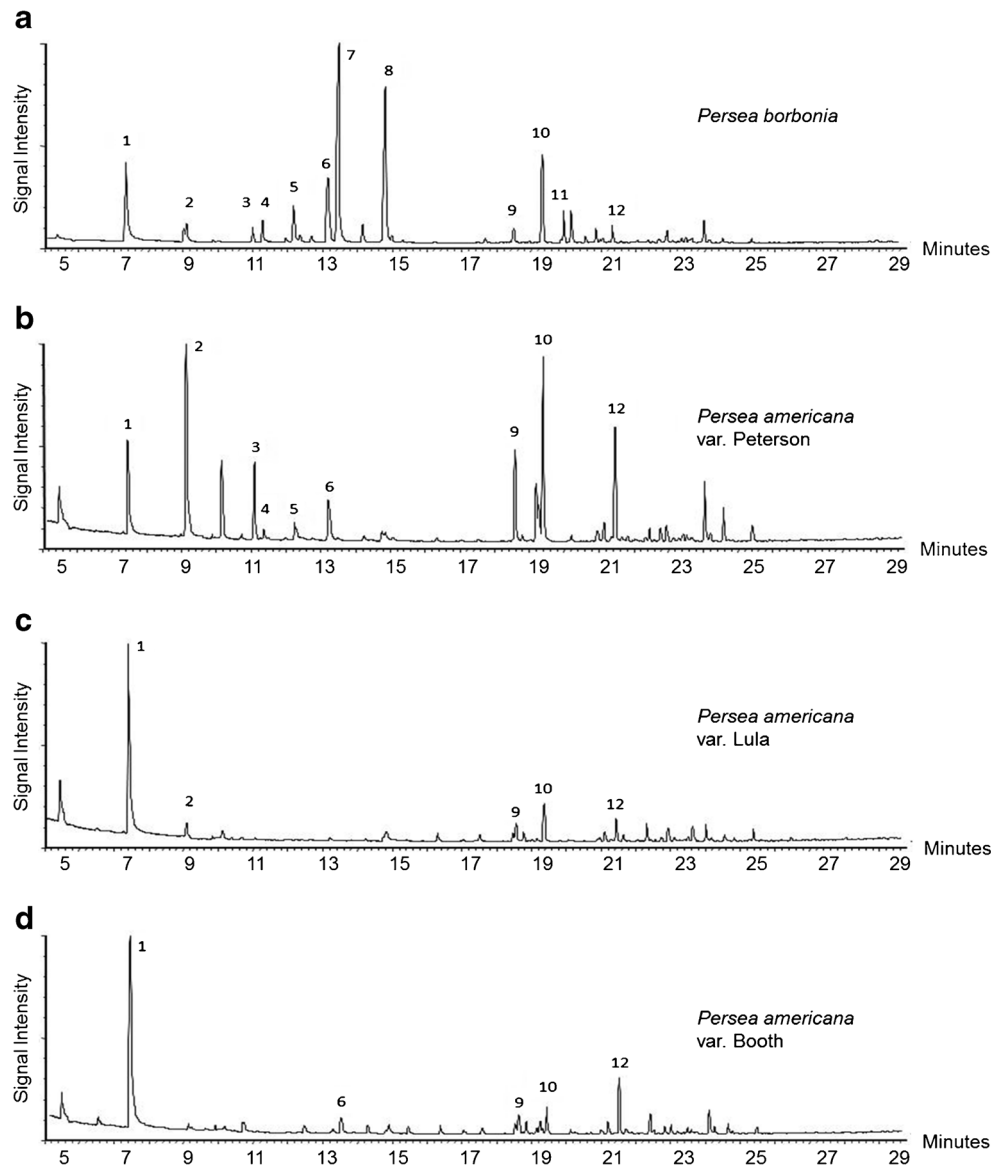
Release Rates of Lures Gravimetric release was calculated for manuka, RB Full, and the Alpha Scents eucalyptol lures (Fig. 4 a–c). Manuka lures released 135.6 ± 5.4 and 121.5 ± 12.3 mg.day⁻¹, for days 1–4 and 8–9, respectively. The decay constant was estimated to be -0.005 with an R^2 of 0.781 (Fig. 4a). Eucalyptol lures had the highest release rates of 1208 ± 132.6 and 1350 ± 173.2 mg.day⁻¹ for days 1–4 and 8–9, respectively. The decay constant was estimated to be -0.013 , with an R^2 of 0.996 (Fig. 4b). Our RB Full lure released 17.7 ± 2.2 mg.day⁻¹ for days 1–4 and 57.2 ± 5.0 mg/day for days 8–9. The decay constant was estimated to be -0.004 , with an R^2 of 0.998 (Fig. 4c).

Beetle Boring Assay We exposed beetles to a boring medium (tightly coiled paper) treated with RB Full, RB-eucalyptol, eucalyptol alone, or negative (untreated) control to determine if these components affected boring of galleries. There was an effect of odor blends on initiation of boring behavior (Fig. 5). During the 4 days of observation, a larger proportion of beetles bored into the eucalyptol treatment than into the negative control (coefficient=1.802, SE=0.583, $z=3.09$, $P=0.002$). There was no difference among the proportion of beetles that bored into the RB-Full (coefficient=0.947, SE=0.564, $z=1.68$, $P=0.093$) and RB-eucalyptol (coefficient=0.850, SE=0.573, $z=1.48$, $P=0.140$) treatments, as compared to the control over this period of time (Fig. 5).

Discussion

Phytochemicals are important for protecting plants against infestation by microbes or invertebrates (Gershenson and Dudareva 2007; Osbourn 1999). However, for specialist insects, these compounds can serve as attractants and may be

Fig. 1 Mass chromatograms of two *Persea* species. Volatile odors of cut wood were identified for redbay (*Persea borbonia*; Panel A), and three varieties of avocado: Peterson (Panel B), Lula (Panel C), and Booth (Panel D). Identified compounds: 1, ethanol; 2, α -pinene; 3, β -pinene; 4, sabinene; 5, myrcene; 6, limonene; 7, eucalyptol; 8, cymene; 9, cubebene; 10, α -copaene; 11, linalool; 12, β -caryophyllene



used to the advantage of the insect (Nishida 2002). Most ambrosia beetle species (95 %) are considered generalists

(Hulcr et al. 2007), but *X. glabratus* is specialized on trees from the family Lauraceae (Hulcr and Lou 2013). Our

Fig. 2 Box plot of *Xyleborus glabratus* captured in traps baited with redbay odor and subtractive blends. Traps were placed in a redbay forest in Wekiwa Springs State Park for 2 week. The total number of *X. glabratus* captured was recorded for each treatment for five replicates. Beetle captures indicated by the same letter are not different ($P < 0.05$)

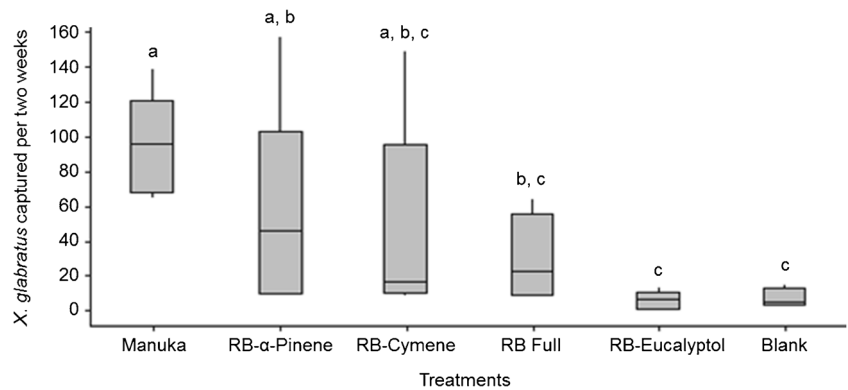


Table 2 Linear mixed effects model analysis for captures of *Xyleborus glabratus*

Factor ¹	Estimate	S.E.	d.f.	t value	P-value
α -pinene	-0.2577245	0.2633712	20	0.978560	0.34
cymene	-0.0511297	0.2633712	20	0.194136	0.85
eucalyptol	1.4402798	0.2633712	20	5.468630	<0.001
manuka	2.4853434	0.3855360	20	6.446462	<0.001
Random effect:	Five trap locations explaining 6 % of the variation				

¹ Contribution of each component to the number of *Xyleborus glabratus* captured on baited traps during the first experiment is displayed above

experiments show that eucalyptol, a major component in some lauraceous hosts, is important for attraction to, and acceptance of, redbay by *X. glabratus*. When testing our synthetic redbay blends, the presence of *p*-cymene did not appear to affect attraction of *X. glabratus*, whereas the presence of eucalyptol was correlated with attraction of *X. glabratus* to traps. The presence of α -pinene in the RB full blend may have resulted in decreased capture of *X. glabratus*; however, this will need to be addressed with greater replication. When we tested a high release of eucalyptol alone, we captured significant numbers of *X. glabratus*, with this catch similar to that found to the known attractant (manuka).

In addition, boring assays indicated that eucalyptol alone increased the rate of beetle boring when compared to negative controls. To our knowledge, this is the first demonstration of a host-specific boring stimulant in an ambrosia beetle species. Ethanol may be a boring stimulant for ambrosia beetle species (Elliott et al. 1983); however, as a product of fermentation, ethanol is not a host-specific compound. The effect of eucalyptol as a

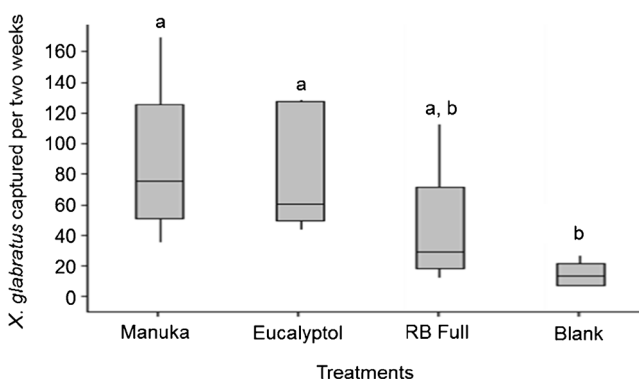


Fig. 3 Box plot of *Xyleborus glabratus* captured in traps baited with redbay odor, manuka oil, eucalyptol, or negative control. Traps were placed in a redbay forest in Wekiwa Springs State Park for 2 weeks. The total number of *X. glabratus* captured was recorded for each treatment for five replicates. Beetle captures indicated by the same letter are not different ($P < 0.05$)

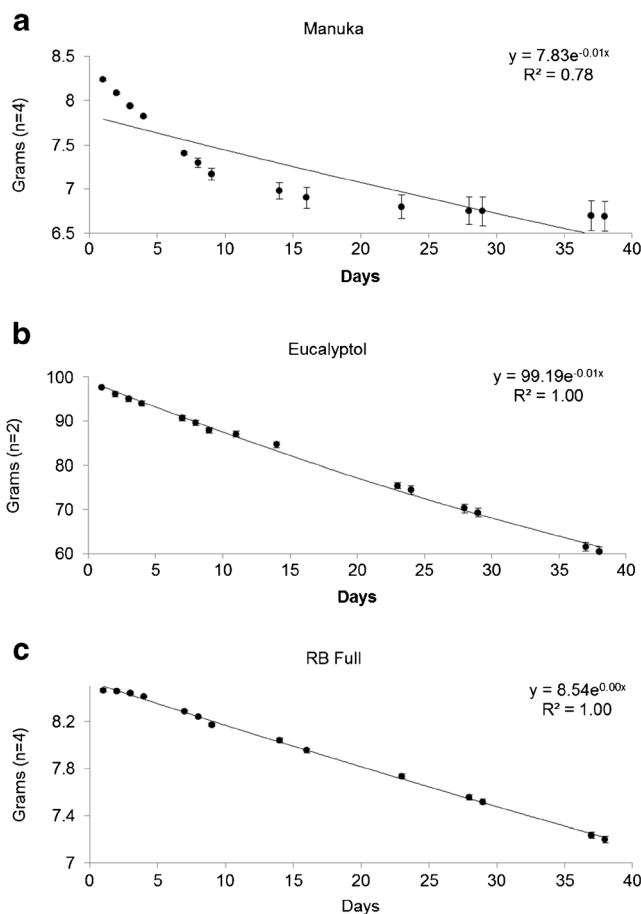


Fig. 4 Release rates of lures. Manuka (panel A), eucalyptol (panel B), and redbay Full Blend (Panel C) lures were deployed outdoors in Lake Alfred, FL, and the gravimetrically measured over 40 d

boring stimulant suggests that *X. glabratus* may specifically infest hosts that release this compound. The boring data also suggest that certain compounds within the RB Full blend may decrease initiation of boring. Overall, the effect of eucalyptol on boring supports the hypothesis that *X. glabratus* may be more specialized than typical ambrosia beetle species with respect to host finding and acceptance (Hulcr and Lou 2013).

Our results have practical implications for management of *X. glabratus* and laurel wilt. First, eucalyptol by itself in high-release devices can be an effective lure for *X. glabratus*. The lures developed by Alpha Scents were characterized by stable release of eucalyptol over 30 days of monitoring; however, we expect that the release should be stable for a longer interval. Based on the extrapolation of the fitted exponential decay equation for the release rate of this lure, the predicted release rate is relatively linear for up to 100 days ($R^2 = 0.976$) and still reasonably linear out to 200 days ($R^2 = 0.898$). Considering the cost of manuka

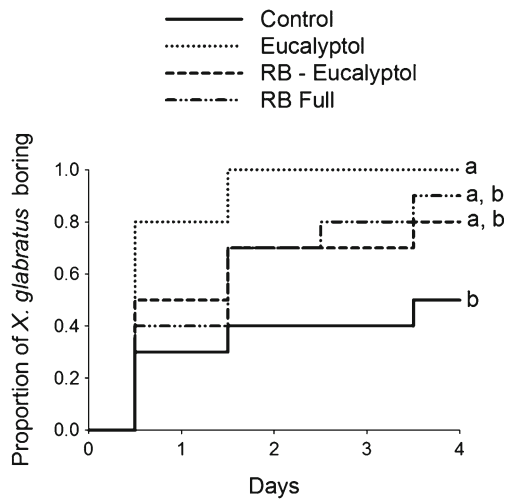


Fig. 5 Boring behavior of *Xyleborus glabratus* on substrate treated with redbay (RB) odors. The proportion of beetles initiating boring each day for each treatment is displayed (dashed line = eucalyptol, dotted line = RB Full, dot dashed line = RB-eucalyptol, solid line: negative control). RB Full, RB-eucalyptol, eucalyptol alone, and water-treated strips of chromatography paper were rolled and placed in BEEM vials. A single beetle was placed into each tube ($N=10$) for each treatment. Presence or absence of boring was noted for each beetle for each day over a 4 day period. Proportions of beetles boring indicated by the same letter within the duration evaluated are not different ($P < 0.05$)

oil, the short effective life-span of manuka oil lures (Hanula et al. 2013), and the equivalent performance of the newly tested high-dosage eucalyptol lures, we believe eucalyptol lures should be an effective alternative to manuka lures for monitoring and other control techniques for this beetle. These lures could be used in either forest or agricultural settings, and may be even more effective in an environment with low eucalyptol background, such as within monocultures of avocado. Additionally, the effectiveness of eucalyptol lures may be increased further when paired with the odors of the beetles' symbiotic fungus, given that the fungal odors increase catch of *X. glabratus* when paired with manuka lures (Hulcr et al. 2011; Kuhns et al. 2014).

It is important to consider that odor preference of *X. glabratus* in the U.S. may have diverged from that of the parent population in Asia. Our data suggest that eucalyptol is critical for host location and acceptance behavior in *X. glabratus*, yet some materials (avocado bolts and manuka lures) used to attract these beetles do not appear to contain much, if any, eucalyptol. In addition, the lures tested in the current investigation omitted certain commercially unavailable or expensive sesquiterpenes that may also be effective attractants of *X. glabratus*. Although it has not been explicitly tested with pure compound, α -copaene is thought to be the major long-range attractant of *X. glabratus* (Hanula and Sullivan 2008;

Kendra et al. 2011). Therefore, it appears that several distinct volatiles may play a role in host selection by this species. Redbay and avocado are not historical components of the natural host assemblage of this invasive beetle. Avocado has been introduced into Asia for crop production, but there are no reports of *X. glabratus* infesting these trees at this time. Odors of North American hosts may differ from odors of hosts with which *X. glabratus* coevolved in its native range. While both avocado and redbay emit odors that are sufficient to attract, it is possible that neither odor blend is optimal for attraction. Some natural hosts have been evaluated for attraction to *X. glabratus*, including *Cinnamomum camphora* (L.) J. Presl., which was found to be attractive to *X. glabratus* (Mayfield et al. 2013). While these authors did not evaluate the eucalyptol content of the *C. camphora* used in their experiments, this species is known to contain significant amounts of eucalyptol (Pélissier et al. 1995). These findings suggest that eucalyptol may be used by *X. glabratus* for host discrimination in its native range.

Although attraction to eucalyptol may have also evolved prior to the introduction of *X. glabratus* into the new world, there is potential for rapid evolution in ambrosia beetles species, through haplodiploidy and inbreeding (Kirkendall 1983). In the case of invasive ambrosia beetles, host selection behavior must reflect the ability of the symbiotic fungus to perform well within the wood of a newly invaded tree species. Therefore, *R. lauricola* may co-evolve to perform well on hosts attractive to the beetle vector and, reciprocally, the beetle may evolve attraction to hosts that are effectively colonized by the fungus. Since eucalyptol is attractive to *X. glabratus*, we predict *R. lauricola* may perform well within hosts that release this chemical.

Eucalyptol was not detected among the volatiles from the three avocado varieties tested, indicating that its production may be lower or absent in avocado. Since eucalyptol increases boring behavior, the lack of eucalyptol in avocado may be a contributing factor to the poor performance of *X. glabratus* observed on avocado (Brar et al. 2013). This may also help explain why *X. glabratus* is seldom found, currently, in avocado groves in south Florida. Chemical surveys of avocado varieties for eucalyptol content may help identify specific varieties attractive to this pest, which may be sub-optimal for cultivation in areas where this beetle species is endemic.

Acknowledgments We thank two anonymous reviewers for their advice that improved a previous version of the manuscript. Jack Smoot and Russell Rouseff provided insights for and assisted with gas chromatography/mass spectrometry. We acknowledge Alice Bard for her help in securing appropriate permits from FDEP. Alpha Scents, Inc. graciously provided prototype lures for field trials. Funding for this research was provided by NIFA (USDA-SCRI) grant number 2009-51181-05915.

References

- Brar GS, Capinera JL, Kendra PE, McLean S, Peña JE (2013) Life cycle, development, and culture of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *Fla Entomol* 96:1158–1167
- Carrillo D, Duncan RE, Peña JE (2012) Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) that breed in avocado wood in Florida. *Fla Entomol* 95:573–579
- Cox DR (1972) Regression models and life-tables. *J R Stat Soc Ser B Methodol* 34:187–220
- Crawley MJ (2009) *The R book*. John Wiley & Sons, Chichester, UK
- Elliott HJ, Madden JL, Bashford R (1983) The association of ethanol in the attack behaviour of the mountain pinhole borer *Platypus subgranosus* Schedl (Coleoptera: Curculionidae: Platypodinae). *Aust J Entomol* 22:299–302
- Evans EA, Crane J, Hodges A, Osborne JL (2010) Potential economic impact of laurel wilt disease on the Florida avocado industry. *Horttechnology* 20:234–238
- Formby JP, Schiefer TL, Riggins JJ (2012) First records of *Xyleborus glabratus* (Coleoptera: Curculionidae) in Alabama and in Harrison County, Mississippi. *Fla Entomol* 95:192–193
- Fraedrich SW, Harrington TC, Rabaglia RJ (2007) Laurel wilt: a new and devastating disease of redbay caused by a fungal symbiont of the exotic redbay ambrosia beetle. *Newsl Mich Entomol Soc* 52:15–16
- Fraedrich SW, Harrington TC, Rabaglia RJ, Ulyshen MD, Mayfield AE, Hanula JL, Eickwort JM, Miller DR (2008) A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. *Plant Dis* 92:215–224. doi:10.1094/pdis-92-2-0215
- Fraedrich SW, Harrington TC, Bates CA, Johnson J, Reid LS, Best GS, Leininger TD, Hawkins TS (2011) Susceptibility to laurel wilt and disease incidence in two rare plant species, pondberry and pondspice. *Plant Dis* 95:1056–1062. doi:10.1094/Pdis-11-10-0841
- Gershenson J, Dudareva N (2007) The function of terpene natural products in the natural world. *Nat Chem Biol* 3:408–414
- Hanula JL, Sullivan B (2008) Manuka oil and phoebe oil are attractive baits for *Xyleborus glabratus* (Coleoptera: Scolytinae), the vector of laurel wilt. *Environ Entomol* 37:1403–1409. doi:10.1603/0046-225x-37.6.1403
- Hanula JL, Mayfield AE, Fraedrich SW, Rabaglia RJ (2008) Biology and host associations of redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae), exotic vector of laurel wilt killing redbay trees in the southeastern United States. *J Econ Entomol* 101:1276–1286
- Hanula JL, Sullivan BT, Wakarchuk D (2013) Variation in manuka oil lure efficacy for capturing *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), and cubeb oil as an alternative attractant. *Environ Entomol* 42:333–340. doi:10.1603/en12337
- Harrington T, Fraedrich S, Aghayeva D (2008) *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. *Mycotaxon* 104:399–404
- Harrington TC, Yun HY, Lu SS, Goto H, Aghayeva DN, Fraedrich SW (2011) Isolations from the redbay ambrosia beetle, *Xyleborus glabratus*, confirm that the laurel wilt pathogen, *Raffaelea lauricola*, originated in Asia. *Mycologia* 103:1028–1036. doi:10.3852/10-417
- Hulcr J, Lou Q-Z (2013) The redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) is a Lauraceae-specialist in its native range: records from the Chinese National Insect Collection. *Fla Entomol* 96:1595–1597
- Hulcr J, Mogia M, Isua B, Novotny V (2007) Host specificity of ambrosia and bark beetles (Col., Curculionidae: Scolytinae and Platypodinae) in a New Guinea rainforest. *Ecol Entomol* 32:762–772
- Hulcr J, Mann R, Stelinski LL (2011) The scent of a partner: ambrosia beetles are attracted to volatiles from their fungal symbionts. *J Chem Ecol* 37:1374–1377. doi:10.1007/s10886-011-0046-x
- Kendra PE, Montgomery WS, Niogret J, Peña JE, Capinera JL, Brar G, Epsky ND, Heath RR (2011) Attraction of the redbay ambrosia beetle, *Xyleborus glabratus*, to avocado, lychee, and essential oil lures. *J Chem Ecol* 37:932–942. doi:10.1007/s10886-011-9998-0
- Kendra PE, Niogret J, Montgomery WS, Sanchez JS, Deyrup MA, Pruett GE, Ploetz RC, Epsky ND, Heath RR (2012) Temporal analysis of sesquiterpene emissions from manuka and phoebe oil lures and efficacy for attraction of *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *J Econ Entomol* 105:659–669. doi:10.1603/Ec11398
- irkendall L (1983) The evolution of mating systems in bark and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Zool J Linn Soc* 77:293–352
- Kuhns EH, Tribuiani Y, Martini X, Meyer WL, Peña J, Hulcr J, Stelinski LL (2014) Volatiles from the symbiotic fungus *Raffaelea lauricola* are synergistic with manuka lures for increased capture of the redbay ambrosia beetle. *Xyleborus glabratus Agric For Entomol* 16:87–94
- Mayfield AE, Hanula JL (2012) Effect of tree species and end seal on attractiveness and utility of cut bolts to the redbay ambrosia beetle and granulate ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). *J Econ Entomol* 105:461–470. doi:10.1603/Ec11348
- Mayfield AE, Peña JE, Crane JH, Smith JA, Branch CL, Ottoson ED, Hughes M (2008a) Ability of the redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) to bore into young avocado (Lauraceae) plants and transmit the laurel wilt pathogen (*Raffaelea* sp.). *Fla Entomol* 91:485–487. doi:10.1653/0015-4040
- Mayfield AE, Smith JA, Hughes M, Dreaden TJ (2008b) First report of laurel wilt disease caused by a *Raffaelea* sp. on avocado in Florida. *Plant Dis* 92:976–976. doi:10.1094/pdis-92-6-0976a
- Mayfield AE, MacKenzie M, Cannon PG, Oak SW, Horn S, Hwang J, Kendra PE (2013) Suitability of California bay laurel and other species as hosts for the non-native redbay ambrosia beetle and granulate ambrosia beetle. *Agr For Ent* 15:227–235
- Niogret J, Kendra PE, Epsky ND, Heath RR (2011) Comparative analysis of terpenoid emissions from Florida host trees of the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *Fla Entomol* 94:1010–1017
- Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. *Annu Rev Entomol* 47:57–92
- Osborn AE (1999) Antimicrobial phytoprotectants and fungal pathogens: A commentary. *Fungal Genet Biol* 26:163–168
- Pélessier Y, Marion C, Prunac S, Bessière J-M (1995) Volatile components of leaves, stems and bark of *Cinnamomum camphora* Nees et Ebermaier. *J Essent Oil Res* 7:313–315
- Peña JE, Carrillo D, Duncan RE, Capinera JL, Brar G, Mclean S, Arpaia ML, Focht E, Smith JA, Hughes M, Kendra PE (2012) Susceptibility of *Persea* spp. and other Lauraceae to attack by redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae). *Fla Entomol* 95:783–787
- Rabaglia RJ, Dole SA, Cognato AI (2006) Review of American *Xyleborina* (Coleoptera: Curculionidae: Scolytinae) Occurring North of Mexico, with an Illustrated Key. *Ann Entomol Soc Am* 99:1034–1056. doi:10.1603/0013-8746
- Ranger CM, Reding ME, Persad AB, Herms DA (2010) Ability of stress related volatiles to attract and induce attacks by *Xylosandrus germanus* and other ambrosia beetles. *Agric For Entomol* 12:177–185
- Walter AJ, Kells SA, Venette RC, Seybold SJ (2010) Boring in response to bark and phloem extracts from North American trees does not explain host acceptance behavior of *Orthotomicus erosus* (Coleoptera: Scolytidae). *Environ Entomol* 39:661–669
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) GLM and GAM for count data. In: *Mixed effects models and extensions in ecology with R*. Springer, pp 209–243