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Attraction of the Redbay Ambrosia Beetle, *Xyleborus glabratus,* to Avocado, Lychee, and Essential Oil Lures

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Abstract The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, is an exotic wood-boring insect that vectors the mycopathogen responsible for laurel wilt, a lethal vascular disease of trees in the Lauraceae. High mortality has occurred in native *Persea* species in the southeastern U.S., and the vector-pathogen complex poses an imminent threat to the production of commercial avocado, *P. americana*, in south Florida. There is a critical need for effective attractants to detect, monitor, and control this invasive pest. This study combined field tests and laboratory bioassays to evaluate the response of female *X. glabratus* to host-based volatiles from wood of avocado (cultivars of West Indian, Guatemalan, and Mexican races); from wood of lychee (*Litchi chinensis,* a presumed non-host that is high in the sesquiterpene α-copaene, a putative attractant); and to commercial lures containing manuka and phoebe oils, two reported attractive baits. Volatile collections and GC-MS analyses were performed to quantify the sesquiterpene content of test substrates. In the field, traps baited with lychee wood captured more beetles than those with wood from avocado cultivars; traps baited with phoebe oil lures captured more beetles than those with manuka oil lures (the current monitoring tool). In field and laboratory tests, *X. glabratus* did not show a preference among avocado races in either attraction or host acceptance (initiation of boring). In choice tests, lychee was more attractive than avocado initially, but a higher percentage of beetles bored into avocado, suggesting that lychee emits more powerful olfactory/visual cues, but that avocado contains more of the secondary cues necessary for host recognition. Emissions of α-copaene, β-caryophyllene, and α-humulene were correlated with field captures, and lychee wood may be a source of additional semiochemicals for *X. glabratus*.

Key Words Avocado · α-Copaene · Gas chromatography-Mass spectroscopy (GC-MS) · *Litchi chinensis* · Lychee · Manuka oil · *Persea americana* · *Persea borbonia* · Phoebe oil · Redbay ambrosia beetle · Sesquiterpene · *Xyleborus glabratus*

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Introduction

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is a minute (~2 mm long) wood-boring insect that vectors the fungus responsible for laurel wilt, a newly-described vascular disease causing high mortality of trees in the Lauraceae in the southeastern United States (Fraedrich et al., 2008). Native to India, Bangladesh, Japan, Myanmar, and Taiwan, *X. glabratus* was first detected in the U.S. in 2002 near Port Wentworth, Georgia (Rabaglia et al., 2006). Since then, the vector-pathogen complex has spread along the coastal plain into South Carolina and Florida, and has been reported from an isolated county in Mississippi (USDA-FS, 2011). The basic biology, ecology, and population dynamics of this exotic invasive species are poorly understood. Like other

ambrosia beetles in the tribe Xyleborini, there is extensive inbreeding and haplodiploid sex determination (Rabaglia, 2002). Males of *X. glabratus* are haploid and flightless, and mate with sibling or parental females (consanguineous polygyny) within the brood galleries. Diploid females emerge from their natal trees and engage in brief dispersal flights to locate and colonize new hosts.

Although not considered a pest in Asia, females of *X. glabratus* are primary colonizers of hosts in the U.S., capable of attacking healthy unstressed trees. During gallery excavation, females introduce spores of several symbiotic fungi (Harrington et al., 2010), including the pathogenic *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Harrington et al., 2008), carried in mycangial pouches located at the base of the mandibles (Fraedrich et al., 2008). The resultant fungal growth (ambrosia) provides food for larvae and adults within the gallery, but the fungus also invades the host vascular system. Similar to the situation with oak wilt (Jacobi and MacDonald, 1980), it is thought that laurel trees infected with *R. lauricola* respond by forming walls within the xylem vessels (parenchymal tyloses) and by secreting gums and resins that block water transport. This results in systemic wilt and ultimately tree death, which can occur in as little as 6 weeks (Mayfield et al., 2008).

Primary host trees in the southeastern U.S. are native *Persea* species, including redbay [*Persea borbonia* (L.) Spreng.], swampbay [*P. palustris* (Raf.) Sarg.], and silkbay (*P. humilis* Nash), but avocado (*P. americana* Mill.) also has been confirmed as a susceptible host (Mayfield et al., 2008). Additional U.S. hosts include sassafras [*Sassafras albidum* (Nuttall) Nees], camphor tree [*Cinnamomum camphora* (L.) J. Presl], spicebush [*Lindera benzoin* (L.) Blume], pondberry [*Lindera melissifolia* (Walter) Blume], and pondspice [*Litsea aestivalis* (L.) Fernald] (Fraedrich et al., 2008), but other woody lauraceous species still need to be evaluated. In Florida, the widespread distribution of hosts, particularly redbay and swampbay, has facilitated a rapid southward spread of the pest complex since its initial detection in the state in 2005. This has been exacerbated by human transport of infested material, particularly firewood (FDACS-DPI, 2010). In February 2011, laurel wilt and breeding populations of *X. glabratus* were detected in swampbay trees in Miami-Dade County (FDACS, 2011), approximately 14.5 km north of commercial avocado groves.

Avocado production in Florida is worth $14 million annually (USDA-NASS, 2011), and replacement costs of commercial and backyard avocado trees in south Florida have been estimated at $429 million (Evans and Crane, 2008). California produces 60% of the U.S. market, with annual production worth $415 million (USDA-NASS, 2011). Mexico is the largest producer worldwide, with 'Hass' production for the 2010–2011 market year forecast to reach 1.24 million metric tons (USDA-FAS, 2010), of which one third is exported, valued at $645 million (UN, 2010). Due to the serious economic threat posed by *X. glabratus*, there is a critical need for effective attractants to detect, monitor, and control the spread of this invasive pest. This is particularly important for early detection at ports of entry in southern California, Mexico, and avocado production areas in South America. Preliminary research has provided no evidence of an aggregation pheromone and no strong attraction to its fungal symbiont, to its frass, or to ethanol (a standard attractant for ambrosia beetles), suggesting that host tree volatiles are the primary semiochemicals utilized by dispersing females (Hanula et al., 2008). Additional studies have identified manuka oil and phoebe oil [essential oil extracts from *Leptospermum scoparium* Forst. & Forst. (Myrtaceae) and *Phoebe porosa* Mez. (Lauraceae), respectively] as attractive baits for field monitoring of *X. glabratus* in South Carolina (Hanula and Sullivan, 2008). Based on comparisons of volatile chemicals emitted from chipped redbay wood, manuka oil, and phoebe oil, Hanula and Sullivan (2008) hypothesized that two sesquiterpenes, α-copaene and calamenene, were likely the primary host attractants.

We evaluated the response of female *X. glabratus* to volatile chemicals released from commercially available manuka and phoebe oil lures, from freshly-cut logs (bolts) of avocado (cultivars representative of the three horticultural races), and from freshly-cut bolts of lychee (*Litchi chinensis* Sonn., Sapindacaeae), a presumed non-host recently found to contain large quantities of α-copaene (Niogret et al., 2011). Studies included two field tests conducted in natural stands of swampbay with known infestations of *X. glabratus,* and several laboratory bioassays to assess attraction and boring behaviors in newly-emerged female *X. glabratus*. In addition, volatile collections and gas chromatography-mass spectroscopy (GC-MS) were performed to quantify the sesquiterpene content of the substrates used in field and laboratory tests. These studies were designed to address three principal questions: (1) Does *X. glabratus* show a preference among the three races of avocado; (2) Are essential oil lures competitive with host avocado wood for capture of *X. glabratus*; and (3) Does α-copaene content account for the patterns of attraction observed with female *X. glabratus*?

Methods and Materials

Field Tests Field trapping experiments were conducted in north-central Florida (Alachua County) at the Lochloosa Wildlife Conservation Area (St. John's River Water Management District). This area consisted of mesic forest

composed of an overstory of slash pine, *Pinus elliottii* Englem., with a mixed middlestory that included numerous swampbay trees exhibiting advanced stages of laurel wilt. Presence of *X. glabratus* and symptomatic *Persea* trees had been observed at this area since 2007 (A. E. Mayfield, personal communication). GPS coordinates for the four corners of the study site were as follows: N29.47416, W82.14974; N29.47354, W82.15018; N29.47673, W82.15099; and N29.47707, W82.15013. Test I was conducted from 7 October to 2 December 2009 and evaluated attraction of *X. glabratus* to six treatments: a commercial manuka oil lure (release rate 50 mg oil/d; Synergy Semiochemicals, Burnaby, BC, Canada), wood bolts from three avocado cultivars ['Simmonds', West Indian race, *P. americana* var. *americana* Mill.; 'Brooks Late', Guatemalan race, *P. americana* var. *guatemalensis* Williams; and 'Seedless Mexican', Mexican race, *P. americana* var. *drymifolia* (Schlect. and Cham.)], bolts from lychee (cultivar unknown), and an unbaited control. Test II was conducted from 5 November to 29 December 2009 (adjacent to test I) and contained a commercial phoebe oil lure (50 mg oil/d; Synergy Semiochemicals) in addition to the six treatments used in test I.

Wood bolts were obtained from the USDA-ARS germplasm collection at the Subtropical Horticulture Research Station (SHRS), Miami, FL, USA 1 day prior to test deployment. At time of collection, the ends of the bolts were coated with wax to prevent desiccation, and then both ends re-cut when used as bait at the start of each test. Trap design consisted of two bolts (5 cm diam. × 15 cm length) wired together and hung vertically with two white sticky panels (23×28 cm, Sentry wing trap bottoms; Great Lakes IPM, Vestaburg, MI, USA) stapled back-to-back to the bottom of the bolts. Paired sticky panels were secured further with several binder clips around the edges. The essential oil lures were deployed in four-unit Lindgren funnel traps (BioQuip, Rancho Dominguez, CA, USA), with a single lure tied to the side of the trap, suspended midway between the second and third funnel. The collection cups were filled with 300 ml of an aqueous solution of 10% propylene glycol (Low-Tox antifreeze; Prestone, Danbury, CT, USA) to retain and preserve captured insects.

Field tests followed a randomized complete block design, with five replicate blocks arranged in a rectangular grid. Each block consisted of a row of traps hung ~2 m high in non-host trees, with a minimum of 10 m spacing between adjacent traps in a row, and with 50 m spacing between rows. Both tests were 8 wk in duration and checked every 2 wk. At each sampling date, the retention solutions and sticky panels were collected, a thin layer (~1 cm) was sawed from the bottom of each bolt (to "renew" release of wood volatiles), the solutions/sticky panels were replaced, and the trap positions were rotated sequentially within each row to minimize positional effects on beetle capture.

All sample collections were sorted in the laboratory at the Subtropical Horticulture Research Station (SHRS), and species of bark and ambrosia beetles were counted, photographed, and stored in 70% ethanol. Specimens removed from sticky panels were soaked overnight in histological clearing agent (Histo-clear II; National Diagnostics, Altanta, GA, USA) prior to storage in alcohol. Beetle identifications were confirmed at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI, Gainesville, FL, USA), and voucher specimens were deposited at both SHRS and FDACS-DPI.

Laboratory Bioassays Behavioral bioassays were conducted from March to April 2010 at the University of Florida (Department of Entomology and Nematology, Gainesville, FL, USA) by using the same germplasm accessions used in field tests. Choice tests were performed primarily to assess relative attraction of substrates; no-choice tests were performed to document boring behaviors of host-seeking females presented with individual substrates. Both bioassays were designed to document behavioral responses over time. Experimental insects were female *X. glabratus* (≤3 days post-emergence) reared from field-collected logs of *P. borbonia* maintained in the laboratory under a 0:24 hr (L:D) photoperiod at 25±2°C (Brar et al. unpublished). Test baits were wood bolts (5 cm diam. × 15 cm length) cut 24 hr prior to testing, with the cut surfaces left uncoated. This allowed for partial wound healing and prevented a sticky surface at the cut site that could potentially immobilize beetles.

Two-choice tests were performed in arenas consisting of rectangular plastic bins (122 cm length, 61 cm width, 16 cm height) with tight-fitting lids containing screen inserts. The full length of the bin floor was lined with paper to provide a rough surface upon which the beetles could walk. Single bolts of lychee and avocado ('Simmonds' cultivar) were placed at opposite ends of the bin, and 20 female *X. glabratus* were introduced at the center and immediately covered with an inverted glass funnel (6 cm diam.). After 10 min acclimation, the beetles were released by gently removing the funnel, and the test was initiated. The number of beetles on each bolt was recorded at 1, 2, 4, 8, 24, and 48 hr. At 48 hr, the number of beetles that had bored completely into the bolts and the location of the bore site were also recorded. Two-choice tests were replicated five times (run concurrently) by using separate arenas, baits, and beetles, and the position of the bolts was alternated between replications.

Arenas for three-choice tests consisted of the rectangular bins described above, but only the center of the bin floor was lined with a paper disk (60 cm diam.). Single bolts of

the three avocado cultivars were placed in random order around the edge of the paper, and 25 female *X. glabratus* were introduced at the center and allowed to acclimate prior to testing (as above). The number of beetles that had bored completely into each of the bolts and the location of the bore sites were recorded after 24 hr. The experiment was replicated five times (run concurrently) by using separate arenas, bolts, and beetles.

No-choice tests were performed in cylindrical plastic arenas (4.4 l buckets) with screened lids. Each bucket was lined with a paper disk (15 cm diam.) and set up to contain one bolt and 10 female *X. glabratus*. Bolt treatments consisted of lychee, the three cultivars of avocado, and live oak, *Quercus virginiana* (Fagaceae), as a non-host control (Fraedrich et al., 2008; Mayfield et al., 2008). For this bioassay, a beetle was scored positive for boring (considered fully committed to the boring behavior) at the point when full insertion of the pronotum (approximately half the body length, 1 mm) was observed. The number of beetles boring and their location on the bolt were recorded at 2, 4, 6, 8, 24, and 48 hr. No-choice tests were replicated five times for each of the five bolt treatments, by using separate arenas, new bolts, and new beetles for each replicate.

Chemical Collection and Analysis Samples for chemical analysis were prepared by manually rasping the outer layers from freshly-cut branches (5.0±0.5 cm diam.) of lychee, avocado, and live oak, by using methods reported previously (Niogret et al., 2011). Branches of lychee and avocado were collected from the same germplasm accessions used in field tests. Samples consisted of the outer bark and cambial tissue (the lateral meristem including the vascular cambium and cork cambium layers that carry the secondary metabolites). Volatile chemicals were collected from rasped wood (6-g samples, 3 replicates per tree) and from essential oil lures (phoebe and manuka, aged 1 wk in the field, 3 replicates per lure) by using Super Q traps (Analytical Research Systems, Gainesville, FL, USA) according to published methods (Heath and Manukian, 1992; Heath et al., 1993). Samples were spread in a cylindrical glass chamber (4.5 cm diam. × 25 cm length for rasped wood samples; 10 cm diam. × 38 cm length for essential oil lures), purified air was introduced into the chamber (1 l/min), and headspace volatiles were collected for 15 min. Super Q traps were cleaned by soxhlet extraction with methylene chloride for 24 hr and dried in a fume hood prior to each use. Volatile chemicals were eluted from the Super Q adsorbent with 200 µl of high purity methylene chloride (99.5% pure; ACROS, Morris Plains, NJ, USA). An aliquot of C_{16} standard (5 µg) was added to each sample for quantitative analysis. All chemical sampling was performed within 2 hr of collecting the branch material.

Chemical extracts were analyzed by using a gas-chromatograph (ThermoQuest Trace GC 2000, Austin, TX, USA). The column was fused silica, 25 m long, 0.25 mm i.d., DB-5MS phase (J&W Scientific, Agilent Technologies, Santa Clara, CA, USA), programmed from 50 to 130°C at 15.0°C/min, then from 130 to 220°C at 10.0°C/min, and then held at 220°C for 4 min. The column used in the gas chromatograph interface to the mass spectrometer (Agilent Technologies 5975B) was 25 m long, 0.25 mm i.d., DB-5MS phase (J&W Scientific, Agilent Technologies), programmed at 40°C for 2 min, then from 40 to 130°C at 10.0°C/min, then from 130 to 220°C at 20.0°C/min, and then held at 220°C for 4 min. Chemicals were identified by using the NIST mass spectral program version 2.0 d and the NIST/EPA/NIH mass spectral library (NIST05) when Reverse Matches and Matches were >950 and >900‰, respectively. For each sesquiterpene, the Kovats Retention Index (RI) was compared with the RI calculated from synthetic chemicals when commercially available [RI=1358, 1391, 1442, 1477 for α-cubebene (Bedoukian Research Inc., Danbury CT, USA), α-copaene (Fluka Analytical, Stenheim, Germany), β-caryophyllene (Sigma Chemical Co., St. Louis, MO, USA), and α-humulene (Sigma), respectively] or with previously published data [δ-elemene (Singh et al., 2007), β-elemene (Hosni et al., 2008; Karlsson et al., 2009), alloaromadendrene (Tokushima et al., 2010), cadinene (Singh et al., 2007; Ibrahim et al., 2010), and calamenene (Ibrahim et al., 2010)].

Statistical Analysis Results from the field tests and three-choice bioassays were analyzed by analysis of variance (ANOVA) with Proc GLM (SAS Institute, 2001) followed by least significant difference test (LSD) for mean separation ($P<0.05$). The Box-Cox procedure, which is a power transformation that regresses log-transformed standard deviations ($y+1$) against log-transformed means ($x+1$), was used to determine the type of transformation necessary to stabilize the variance prior to analysis (Box et al., 1978). Paired *t*-tests were used to analyze results from the two-choice bioassays, and regression analysis was used to document patterns in boring behaviors observed in the no-choice bioassays (Systat Software, 2006a). For each sesquiterpene, the captures of *X. glabratus* in field test II were compared to the quantity of chemical per substrate by using Pearson product moment correlation (Systat Software, 2006b). Unless otherwise noted, results are presented as mean ± s.d.; probability was considered significant at a critical level of $\alpha=0.05$.

Results

Field Tests Population levels were low at the study site, and the combined trapping results from both field tests

totaled ~600 ambrosia beetles. *Xyleborus glabratus* was the dominant species, comprising 75.4 and 86.2% of the captures in tests I and II, respectively. Non-target captures included 12 species representing four tribes of ambrosia beetles. Data on the diversity of ambrosia beetles captured with lychee, avocado, and essential oil lures are presented elsewhere (Kendra et al., 2011).

In field test I (Fig. 1a), there were differences in capture of female *X. glabratus* among the six treatments

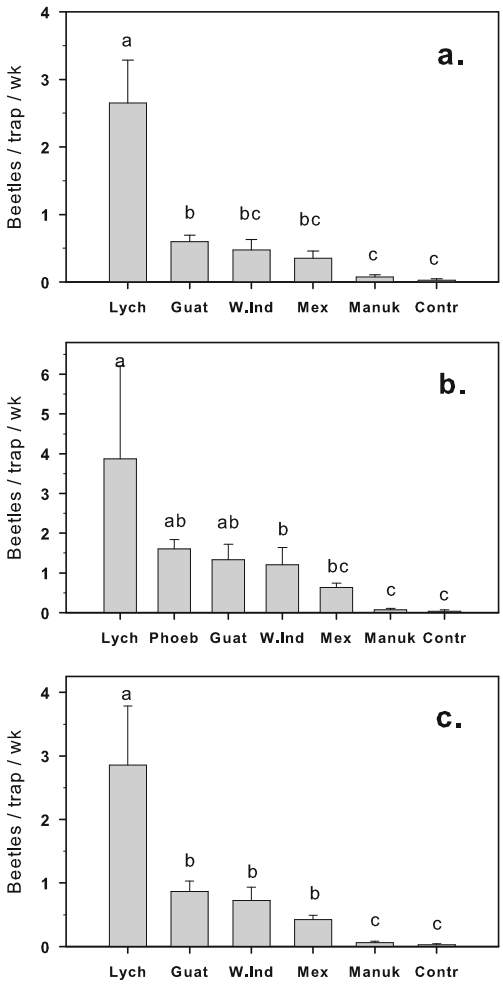

Fig. 1 Mean (±s.e.) captures of female *Xyleborus glabratus* in 8-wk field tests conducted in north-central Florida. (**a**) Test I (N=5) evaluated captures with a manuka oil lure (Manuk); with wood bolts of lychee, *Litchi chinensis* (Lych); and with wood bolts of three cultivars of avocado, *Persea americana*: 'Brooks Late', Guatemalan race (Guat); 'Simmonds', West Indian race (W. Ind); and 'Seedless Mexican', Mexican race (Mex). (**b**) Test II (N=5) evaluated the same treatments as Test I, but also included a phoebe oil lure (Phoeb). (**c**) Combined analysis of the in-common treatments from tests I and II (N=10; There were no interactions between test and treatment for beetle captures, nor an effect caused by test). Commercial lures were deployed in 4-unit Lindgren funnel traps; wood bolts were deployed with sticky traps. Both tests included a control (Contr) treatment that consisted of an unbaited sticky trap. Bars topped with the same letter are not significantly different (LSD mean separation on square root [x+0.5]-transformed data, nontranformed means presented, P<0.05)

(F=16.67; df=5, 29; P<0.001). Traps baited with lychee bolts caught significantly more beetles than any other treatment. Captures with avocado bolts were intermediate, and there were no differences observed among the three cultivars evaluated. Manuka-oil-baited traps captured the fewest beetles, but captures were not significantly different from traps baited with the Mexican or West Indian avocado cultivars. Manuka lure-baited traps captured *X. glabratus* during the first 2 wk only; no beetles were captured with manuka lure-baited traps from wk 3 to 8, despite capture of beetles with all other treatments.

Mean captures of *X. glabratus* were slightly higher during the second field test (Fig. 1b), and there were differences in captures among the seven treatments (F=6.51; df=6, 34; P<0.001). Overall, the hierarchy of captures was similar to that observed with test I. Lychee bolts captured the greatest number of beetles, although not significantly different from captures with the phoebe lure or the Guatemalan avocado. The phoebe lure was also competitive with bolts from all three avocado cultivars. All beetle captures with manuka lure-baited traps occurred within the first 2 wk of the test.

Two-way ANOVA indicated there were no interactions between test and treatment for captures of *X. glabratus* (F=0.58; df=5, P=0.714), nor was there an effect caused by test (F=0.81; df=1, P=0.373). Therefore, data from the two field tests were combined, generating 10 replicate blocks of the six in-common treatments. With the increase in number of observations, there were differences in captures among treatments (F=18.26; df=5, 59; P<0.001) and improved separation of means (Fig. 1c). Captures with lychee bolts were significantly greater than captures with avocado bolts, and captures with avocado were significantly greater than captures with manuka lure, which was comparable to the unbaited control treatment.

Laboratory Bioassays In two-choice tests with bolts of lychee and avocado (Fig. 2), more *X. glabratus* were attracted to lychee at the initial 1-hr and 2-hr observations (t=−2.49, df=8, P=0.038; and t=−2.41, df=8, P=0.043, respectively). By 4 hr, there were equal numbers of beetles on both choices (t=−0.30, df=8, P=0.770). By 8 hr, many of the beetles had left the lychee, but the numbers on avocado remained unchanged. By the end of the 48-hr test, the pattern had reversed and there were more *X. glabratus* on avocado (t=3.236, df=8, P=0.012). With both lychee and avocado, the majority of *X. glabratus* were actively boring into the bolts for all observations taken at or beyond 8 hr. At 48 hr, there were more beetles boring on avocado than on lychee (t=2.467, df=8, P=0.039). A higher percentage of boring occurred on the cut ends of the bolts as compared to the bark surfaces (91.3 and 80.0% for avocado and lychee, respectively).

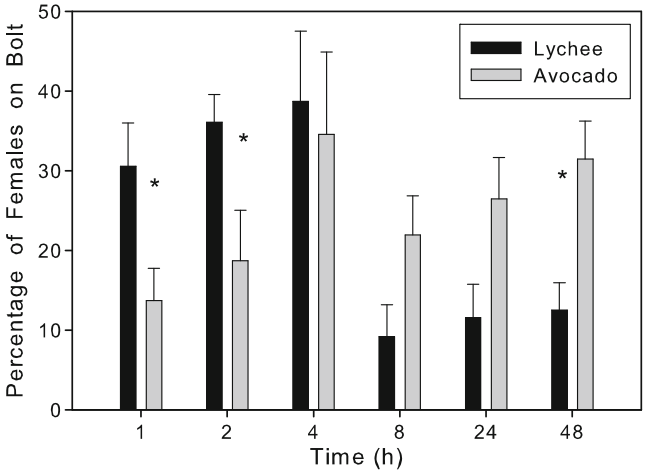

Fig. 2 Mean (±s.e.) percentage of female *Xyleborus glabratus* on wood bolts of lychee, *Litchi chinensis*, or avocado, *Persea americana* cv. 'Simmonds', West Indian race, presented in a 48-hr two-choice bioassay. With both choices, the majority of beetles were boring into the bolts at readings ≥8 hr. Asterisk indicates that mean responses to lychee and avocado are significantly different (*t*-test, $P<0.05$)

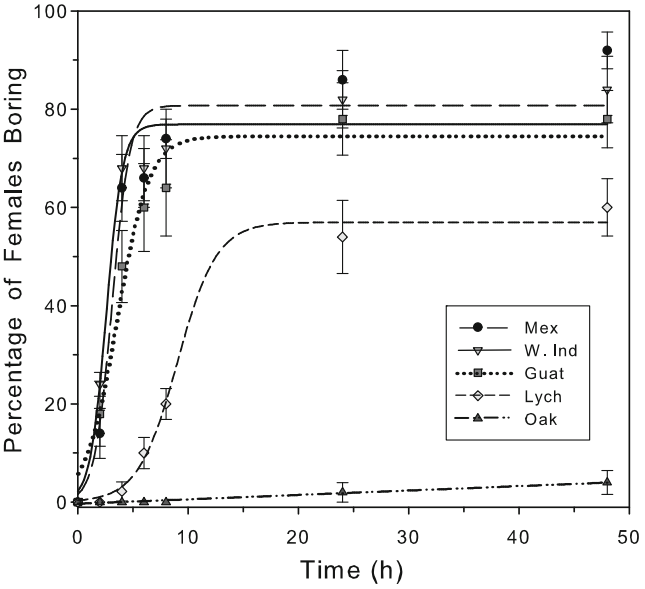

Fig. 3 Mean (±s.e.) percentage of female *Xyleborus glabratus* boring into wood bolts presented in a 48-hr no-choice bioassay. Treatments consisted of three cultivars of avocado, *Persea americana*: 'Brooks Late', Guatemalan race (Guat), 'Simmonds', West Indian race (W. Ind), and 'Seedless Mexican', Mexican race (Mex); lychee, *Litchi chinensis* (Lych); and Live oak, *Quercus virginiana* (L. Oak). Rate of boring was best fit by sigmoidal regression models (see text)

In three-choice tests among the avocado cultivars, the percentage of *X. glabratus* boring into each cultivar after 24 hr was as follows: 31.2 (±16.5)% West Indian; 28.6 (±20.3)% Guatemalan; and 40.2 (±7.0)% Mexican. There was not a significant difference among choices ($F=0.755$; $df=2, 14$; $P=0.491$). The percentage of boring on cut ends of avocado bolts (all cultivars combined) was 64.5%.

The composite results of no-choice bioassays with different bolt treatments are presented in Fig. 3. Regression analysis with sigmoidal models best described the relationships between the time after presentation of avocado or lychee bolts and the percentage of female *X. glabratus* engaged in boring behaviors [Mexican: $y = 80.75/\left(1+e^{-[(x-3.13)/0.80]}\right)$, $R^2=0.951$; West Indian: $y = 76.92/\left(1+e^{-[(x-2.53)/0.76]}\right)$, $R^2=0.971$; Guatemalan: $y = 74.47/\left(1+e^{-[(x-3.56)/1.44]}\right)$, $R^2=0.973$; and lychee: $y = 56.99/\left(1+e^{-[(x-9.04)/1.78]}\right)$, $R^2=0.995$]. Boring was initiated quickly with the avocado cultivars, with the maximum percentage reached by ~8 hr. With lychee bolts, females spent more time walking on the wood substrate before choosing a site and committing to boring. This resulted in a delay (relative to avocado) in boring response with the maximum percentage not reached until ~15 hr. At the conclusion of the test, the mean percentage of boring was as follows: 80.75 (±5.16)% Mexican; 76.92 (±3.42)% West Indian; 74.47 (±3.84)% Guatemalan; and 56.99 (±1.65)% lychee. Few beetles responded to the bolts of live oak (4.0±1.09%), and the responses observed were likely to be thigmotactic rather than olfactory or gustatory-guided behaviors related to host selection. Response of *X. glabratus* to live oak was best fit by linear regression ($y=-0.326+0.09x$, $r^2=0.976$). At 48 hr, there were differences in boring among the five bolt treatments ($F=32.863$, $df=4, 24$; $P<0.001$). There was no difference in percentage of boring among the three avocado cultivars. Percentage of boring in avocado was greater than in lychee, and boring in live oak was significantly less than in all other treatments. The percentage of boring on cut ends (all bolt treatments combined) was 71.9%.

Chemical Analysis A total of 29 volatile chemicals (detected at quantities ≥0.5 μg) were isolated and identified by Super Q collections and GC-MS analysis. Of these, there were nine sesquiterpenes found in common (Table 1). Sesquiterpene content varied both qualitatively and quantitatively among the samples (Fig. 4), but only three chemicals were positively correlated with captures of *X. glabratus* in field test II. These included α-copaene (*Pearson coeff.*=0.427, $P=0.010$; Fig. 4, peak 3), β-caryophyllene (*Pearson coeff.*=0.395, $P=0.019$; Fig. 4, peak 5), and α-humulene (*Pearson coeff.* = 0.514, $P=0.002$; Fig. 4, peak 6). Volatiles from lychee wood (Fig. 4a) contained large amounts of all three chemicals correlated with beetle captures, but also contained a large peak (identification unknown; RI=1504, quantity=9.21±2.10 μg) not detected in other samples. Wood from the West Indian avocado (Fig. 4c) was lower in α-copaene compared to the other two cultivars. Wood from live oak (Fig. 4e) was low in both α-copaene and α-humulene, but had moderate levels of β-caryophyllene. Calamenene was not correlated with beetle

Table 1 Quantity of volatile sesquiterpenes emitted from wood bolts of lychee, *Litchi chinensis*, three cultivars of avocado, *Persea americana*, and from commercial lures of phoebe oil and manuka oil

Chemical	RI[a]	Lychee	Avocado Cultivars			Live Oak	Essential Oil Lures	
			Guatemalan 'Brooks Late'	West Indian 'Simmonds'	Mexican 'Seedless Mex.'		Phoebe[b]	Manuka[b]
δ-Elemene[c]	1347	0.13±0.09	0.87±0.47	0.67±0.14	0.22±0.04	0.26±0.05	0.10±0.03	0.01±0.00
α-Cubebene[d]	1361	0.41±0.03	11.43±1.82	2.26±0.71	1.28±0.25	0.10±0.07	0.55±0.18	3.68±0.22
α-Copaene[d,e]	1394	23.61±3.46	12.93±4.99	2.81±0.60	15.95±4.95	1.06±0.75	8.62±2.76	4.82±0.18
β-Elemene[c]	1403	0.23±0.08	4.09±1.44	1.24±0.33	1.69±0.41	1.27±0.26	0.63±0.17	0.91±0.05
β-Caryophyllene[d]	1443	15.79±4.66	14.39±7.63	1.83±0.44	5.16±0.82	5.18±0.30	0.20±0.08	1.59±0.19
α-Humulene[d]	1477	3.58±0.54	1.30±0.66	0.38±0.09	0.39±0.07	0.18±0.10	0.44±0.11	0.15±0.03
Alloaromadendrene[c]	1482	0.24±0.09	3.55±1.81	0.03±0.01	0.78±0.20	1.61±0.46	1.14±0.11	0.35±0.02
Cadinene[c]	1530	2.28±0.52	1.61±0.74	0.80±0.10	2.98±0.82	0.82±0.34	1.44±0.12	0.50±0.05
Calamenene[c]	1537	0.15±0.01	1.60±0.74	0.04±0.00	0.12±0.10	0.10±0.06	0.33±0.03	5.88±0.38

Table entries are in μg (mean ± s.d.)

Volatiles were isolated from 6 g of rasped wood samples by Super Q collection and then analyzed by GC-MS (DB-5MS column)

[a] Mean Kovats Retention Index calculated from 3 replicate runs per sample

[b] Emissions measured from lures 1 wk old.

[c] Identification based on comparison with RI from published reports (see text)

[d] Identification based on comparison with RI from synthetic chemicals (see text)

[e] Co-eluted with ylangene

captures and was a major component only in the manuka lure sample (Fig. 4g, peak 9).

Discussion

The current detection and monitoring system for *X. glabratus* in the United States is the Lindgren funnel trap baited with manuka oil lures (Lindgren, 1983; CISEH, 2010). Results from our field tests indicated that manuka lures were not competitive with the volatiles released from host avocado wood. In the original studies conducted by Hanula and Sullivan (2008), neat manuka oil dispensed from glass vials was as attractive to *X. glabratus* as wood bolts from redbay. The commercial lure evaluated in our tests released ~50 mg/d of manuka oil (total oil loss determined gravimetrically, per manufacturer specifications). This may not be adequate for extended capture of *X. glabratus*, since no beetles were caught with manuka lures after 2 wk in either test. Two additional field tests conducted in 2010 (Kendra et al. unpublished) confirmed a maximum field life of 2–3 wk for this lure in Florida. Further work is needed to determine the effective loading dosage of manuka oil as well as the release rates of individual attractive components (i.e., α-copaene, calamenene), as has been quantified for other insect lures (Heath et al., 2007). Published chemical analyses of manuka oil have shown a high degree of variability among extracts from trees of different geographic regions in New Zealand (Porter and Wilkins, 1998), which may also contribute to variability in attraction of *X. glabratus.*

Commercial phoebe oil lures were competitive with host avocado wood for capture of *X. glabratus*, and were effective throughout the entire 8-wk test. Unfortunately, the source of phoebe oil, the Brazilian walnut tree, is a limited resource in the Amazon rain forest and may not be available for future use. Another drawback of both manuka and phoebe lures was their non-specificity for *X. glabratus*. Ambrosia beetles are a large, diverse faunal group in tropical and subtropical regions (e.g., >500 recognized *Xyleborus* spp. from the neotropics, Africa, and Asia; Rabaglia, 2002), and numerous non-target species were captured in traps baited with essential oil lures in north Florida (Kendra et al., 2011). Once healthy swampbay trees were attacked by *X. glabratus*, the stressed trees were susceptible to further attack by an array of secondary colonizers (primarily species from within the tribe Xyleborini). These secondary species were attracted to the same host-based volatiles as *X. glabratus*, contributing to high non-target captures in field traps (14, 25, and 85% non-target captures at three field sites in Alachua and Marion Counties, FL). A similar diversity of ambrosia beetles (~15 species) has been observed to attack stressed avocado trees in south Florida (Peña et al. unpublished), and manuka-baited Lindgren traps deployed in avocado groves (Miami-Dade County, FL, USA) captured high numbers of

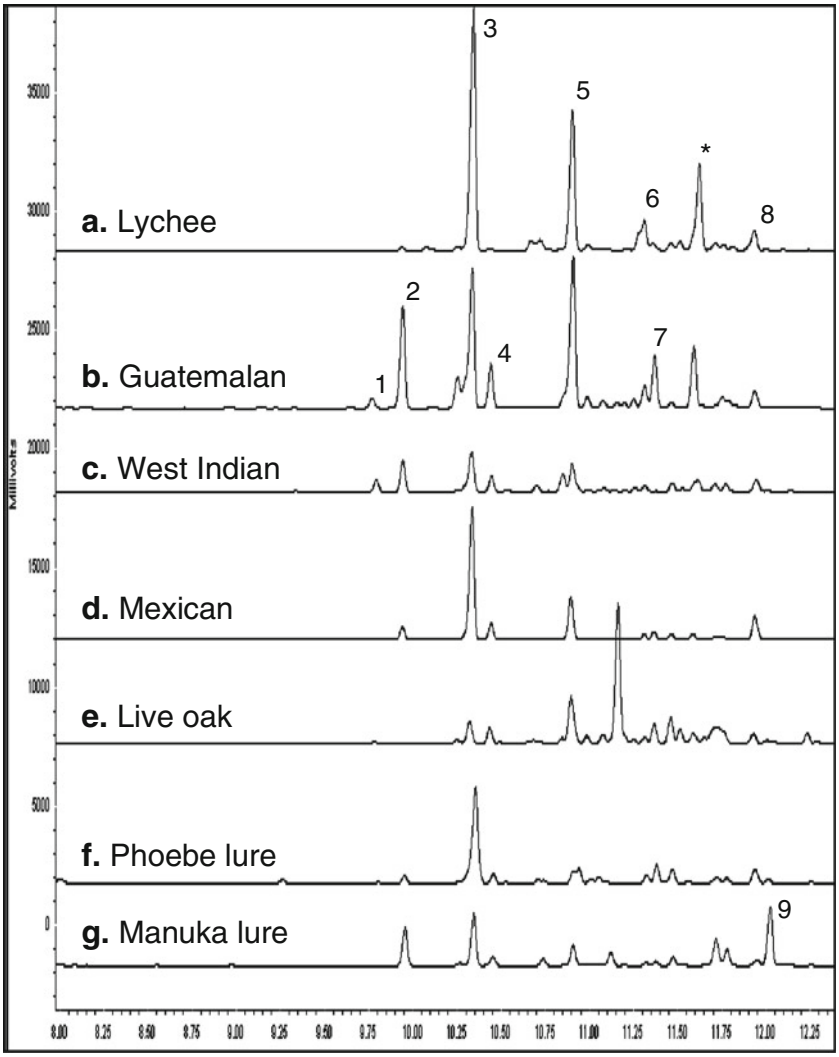

Fig. 4 Representative GC-MS analyses of sesquiterpenes obtained by Super Q collections from wood of (**a**) lychee, *Litchi chinensis*, from wood of three cultivars of avocado, *Persea americana*: (**b**) 'Brooks Late', Guatemalan race, (**c**) 'Simmonds', West Indian race, and (**d**) 'Seedless Mexican', Mexican race; from wood of (**e**) live oak, *Quercus virginiana*, and from commercial lures of (**f**) phoebe oil and (**g**) manuka oil. Peak identifications are as follows: 1) δ-elemene, 2) α-cubebene, 3) α-copaene, 4) β-elemene, 5) β-caryophyllene, 6) α-humulene 7) alloaromadendrene, 8) cadinene, 9) calamenene, and * unidentified chemical (RI=1504)

Ambrosiodmus obliquus (LeConte) and *Hypothenemus* spp. (Kendra et al., 2011).

Two trap types were used in our field tests evaluating captures of *X. glabratus* with attractive substrates: funnel traps with the formulated lures of manuka and phoebe oil; and sticky traps with the wood bolts of avocado and lychee. In an initial field test conducted in Marion County, FL, USA (Kendra et al., 2011), few ambrosia beetles were captured with funnel traps baited with wood bolts. At the recommendation of A. E. Mayfield (USDA-Forest Service, Asheville, NC, USA), we changed our trap design to a sticky panel for evaluation of host wood in the current tests. However, in our experiments, funnel traps were used for deployment of commercial lures rather than sticky panel traps because our goal was to evaluate the efficacy of the current monitoring tool for detection of *X. glabratus.*

Our study provided no evidence that female *X. glabratus* show a preference among the horticultural races of avocado. There were no differences in attraction to bolts from the three cultivars presented in field tests nor in the three-choice bioassays, despite differences in chemical profiles evident from GC analysis. Nor was there a difference in percentage of boring (interpreted as host recognition/acceptance) among the cultivars presented in no-choice tests. Mayfield et al. (2008) evaluated four different cultivars ('Hass', Guatemalan-Mexican hybrid; 'Monroe', Guatemalan-West Indian hybrid; 'Winter Mexican' Mexican; and 'Catalina', Guatemalan-West Indian hybrid) as young potted trees in no-choice tests and found no differences in boring of *X. glabratus* or in transmission of the *Raffaelea* pathogen. As a generalist on woody hosts in the Lauraceae, *X. glabratus* did not appear to distinguish among differences in avocado genotypes (intraspecific variation in secondary metabolites). In all of our laboratory bioassays (and in avocado bolts deployed in field tests), we observed that the majority of boring took place along the cut surface of the bolts, suggesting that host trees would be most susceptible to attack by *X. glabratus*

immediately following pruning activities, when there would likely be high release of host volatiles.

Of the wood substrates evaluated, lychee was the most attractive to dispersing female *X. glabratus,* outcompeting avocado in field tests. In two-choice bioassays, lychee bolts were more attractive than avocado initially, but over time a higher percentage of females bored into avocado bolts. This response suggests that lychee wood provides an initially more powerful olfactory/visual cue than avocado; but after settling on lychee, it may be a suboptimal substrate, and thus the beetles continue to forage until they find a more suitable host (avocado) for long-term settling based on other sensory modalities, including gustation. However, when not given a choice, more than half of the females tested did bore into lychee bolts. Although not in the family Lauraceae (like all confirmed U.S. hosts of *X. glabratus*), lychee should be evaluated for host status and susceptibility to laurel wilt. Since lychee is another subtropical fruit tree grown in the same areas as commercial avocado groves, lychee could serve as a potential reservoir for *Raffaelea* even if it is not susceptible to laurel wilt. The latter would not be unexpected since lychee is native to Southeast Asia and occurs sympatrically with endemic populations of *X. glabratus*. In its native regions, *X. glabratus* is not restricted to the family Lauraceae, and has been recorded from hosts in the Dipterocarpaceae, Fagaceae, and Fabaceae, in addition to Lauraceae (Rabaglia et al., 2006).

Chemical analysis of our test substrates identified three sesquiterpenes that were correlated positively with field captures of *X. glabratus*—α-copaene, β-caryophyllene, and α-humulene. β-Caryophyllene and α-humulene (synonymous with α-caryophyllene) have not been evaluated previously for *X. glabratus*, and need further study as potential attractants. The high levels of α-copaene measured from lychee wood support the conclusion of Hanula and Sullivan (2008) that this sesquiterpene functions as a primary host attractant. The same authors identified calamenene as an attractant in native redbay trees; however, calamenene was detected at low levels in our wood substrates (particularly the West Indian avocado) and did not appear to play a role in attraction to avocado or lychee. Quantification of calamenene was also low in the phoebe oil lure that had been aged in the field for 1 wk. Direct evaluations of α-copaene and calamenene as field lures have yet to be done, due to lack of commercial availability in quantities suitable for field tests. Interestingly, α-copaene, β-caryophyllene, and α-humulene have also been implicated as potential attractant semiochemicals for males of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) (Niogret et al., 2011, and references therein). In addition to sesquiterpenes, we detected two monoterpenes, α- and β-pinene, in significant quantities from the avocado cultivars, but not from lychee or live oak (data not shown). Pinenes are known attractants for other species in the Xyleborini, including *Xyleborus pubescens* Zimmermann (Miller and Rabaglia, 2009) and *Xyleborinus saxesenii* (Ratzeburg) (Petrice et al., 2004), and may function as host (Lauraceae) attractants for *X. glabratus* as well.

The unique peak detected in lychee (RI=1504) also needs further evaluation. Unfortunately, the chemical could not be identified even tentatively in this study due to lack of consistent matches in the NIST mass spectral library. Further work is needed for structural confirmation of the chemical and for evaluation of its potential role as an attractant (or possible synergist to α-copaene). Insight into potential attractants for *X. glabratus* may be gained from comparative studies of substrates like lychee and known hosts, including analysis of sesquiterpene content and evaluation of relative attractiveness in field tests or bioassays. However, identification of specific attractants could be facilitated by coupled electroantennography-chromatographic separation (electroantennal detection or EAD) of host volatiles. Future research efforts will be directed toward applying EAD techniques to *X. glabratus* for evaluation of α-copaene, calamenene, β-caryophyllene, α-humulene, and the unidentified peak from lychee.

In summary, our study revealed that the standard detection system for *X. glabratus* (Lindgren funnel trap baited with manuka oil lures) is suboptimal, apparently due to limited longevity of the field lure. Phoebe oil lures were more effective, but their future availability is uncertain. Female *X. glabratus* showed no preference among avocado cultivars (West Indian, Mexican, and Guatemalan races) and preferred to bore into freshly-cut surfaces. This suggests that avocado trees would be most susceptible to attack immediately following pruning activities. Chemical analysis of wood substrates provided supporting evidence that α-copaene is a primary host attractant. Volatiles from lychee wood were more attractive to *X. glabratus* than volatiles from host avocado wood, and lychee may be a source of novel attractants for development of improved detection and control strategies for *X. glabratus*.

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