Evaluation of Litchi chinensis for Host Status to Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae) and Susceptibility to Laurel Wilt Disease

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EVALUATION OF *LITCHI CHINENSIS* FOR HOST STATUS TO *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE) AND SUSCEPTIBILITY TO LAUREL WILT DISEASE

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ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring pest that vectors *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Ophiostomatales: Ophiostomataceae), the etiologic agent of laurel wilt. To date, all confirmed U.S. hosts of *X. glabratus* and suscepts of laurel wilt are members of the family Lauraceae. However, in previous research, an unknown variety of lychee, *Litchi chinensis* Sonn. (Sapindales: Sapindaceae), was found to be highly attractive to *X. glabratus* and elicited boring behaviors. Therefore, a study was undertaken to evaluate two commercial cultivars of lychee, 'Brewster' and 'Mauritius', for susceptibility to attack by *X. glabratus*, for transmission of *R. lauricola*, and for development of laurel wilt disease. In no-choice laboratory bioassays, 35 and 44% of females bored into cut bolts of 'Mauritius' and 'Brewster', respectively. Similar boring was observed on the trunks of two live 'Brewster' trees; but after 3 mo, there was no evidence of beetle reproduction, no symptoms of laurel wilt, and no recovery of *R. lauricola* from tissue associated with beetle galleries. Lychee trees artificially inoculated with an isolate of *R. lauricola* (RL4) that kills lauraceous hosts of this pathogen were asymptomatic after 1 mo, and assays for *R. lauricola* were negative. Chemical analysis indicated that lychee emits several sesquiterpene constituents in common with the Lauraceae, but at lower levels. Based on these data, we conclude that *L. chinensis*, although attractive to female *X. glabratus*, is not a likely reproductive host. This may be due to the inability of lychee wood to support growth of *R. lauricola*, the presumed primary nutritional symbiont of *X. glabratus*.

Key Words: Redbay ambrosia beetle, lychee, laurel wilt, *Litchi chinensis*, *Raffaelea lauricola*

RESUMEN

El escarabajo del laurel rojo, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), es un barrenador exótico que actúa como vector de *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Ophiostomatales: Ophiostomataceae), el agente causal del secamiento del laurel. Hasta el presente, todos los hospederos de *X. glabratus* en USA han sido miembros de la familia Lauraceae. Sinembargo, en investigaciones realizadas anteriormente, se encontró que *X. glabratus* era muy atraido a litchi, *Litchi chinesis* Sonn. (Sapindales: Sapindaceae), y que el escarabajo mostraba comportamiento de barrenador de esta planta. En consecuencia, se comenzaron estudios para evaluar la susceptibilidad de dos variedades de litchi, "Brewster" y "Mauritius" al ataque de *X. glabratus*, la transmissión de *R. lauricola* y el desarrollo de la enfermedad del laurel. En pruebas de laboratorio tipo no-elección, el 35 y 44% de hembras barrenaron secciones de troncos de "Mauritius" y "Brewster". Similarmente, se observó barrenamiento de tallos de dos arboles de "Brewster", pero después de 3 meses, no hubo evidencia ni de reproducción del escarabajo, ni de síntomas de secamiento del laurel, ni se aisló *R. lauricola* del los tuneles perforados por el escarabajo. Arboles de litchi fueron inoculados artificialmente con una cepa virulenta de *R. lauricola* (RL4) pero después de un mes no mostraron sintomas de la enfermedad y pruebas de laboratorio demostraron que no se recuperó *R. lauricola* de estos tejidos. Análisis de laboratorio indicaron que la madera del litchi emite en niveles bajos, varios volátiles de sequiterpenos los cuales son tambien comunes a los encontrados en plantas de la familia Lauraceae. Basados en estos datos, concluímos que aunque las hembras de *X. glabratus* son bastante atraidas a *L. chinensis*,

este no es un hospedero donde se puedan reproducir. Esto es debido aparentemente a que *R. lauricola* que aprentemente es el simbionte nutricional primario de *X. glabratus* no puede crecer en la madera de litchi.

Palabras Clave: barrenador del laurel rojo, litchi, secamiento del laurel, *Litchi chinensis, Raffaelea lauricola*

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring pest that impacts forest ecosystems and agriculture in the United States. During gallery excavation, females of *X. glabratus* introduce conidia of several symbiotic fungal species, including a confirmed pathogen, *Raffaelea lauricola* T. C. Harr., Fraedrich & Aghayeva (Ophiostomatales: Ophiostomataceae) (Fraedrich et al. 2008; Harrington et al. 2008, 2010). The fungi proliferate within the galleries, providing food for the beetle, but presence of *R. lauricola* in American hosts elicits secretion of resins and formation of extensive walls (parenchymal tyloses) within xylem vessels (Inch et al. 2011; Ploetz et al. 2012), culminating in a lethal disease known as laurel wilt. This vascular disease has decimated large populations of native redbay and swampbay (*Persea borbonia* (L.) Spreng. and *P. palustris* (Raf.) Sarg., respectively) along the Atlantic Coastal Plain (Fraedrich et al. 2008), and laurel wilt currently threatens avocado (*P. americana* Mill.) in south Florida (FDACS 2012). First detected in Georgia in 2002, the beetle vector is now established in six southeastern states (USDA-FS 2013), and the laurel wilt epidemic continues to spread (recently reviewed by Kendra et al. 2013).

To date, 12 U.S. species–all in the angiosperm family Lauraceae–have been reported as hosts of *X. glabratus* and/or have shown susceptibility to laurel wilt in nature or through laboratory inoculations with *R. lauricola* (Fraedrich et al. 2008; Hanula et al. 2008; Mayfield et al. 2008, 2013; Mayfield & Hanula 2012; Ploetz & Konkol 2013). Chemical ecology studies provide evidence that dispersing beetles locate appropriate hosts based on their volatile emissions; the current hypothesis is that females respond to a blend of terpenoid compounds (a generalized 'laurel bouquet'), with α-copaene as the primary long-range attractant (Hanula & Sullivan 2008; Niogret et al. 2011a; Kendra et al. 2011, 2012a, 2012c; Hanula et al. 2013). In early studies, freshly-cut logs (bolts) of lychee (*Litchi chinensis* Sonn., Sapindales: Sapindaceae) were evaluated as a positive control for attraction of *X. glabratus* (Kendra et al. 2011). Lychee wood was chosen based on its high content of α-copaene (Niogret et al. 2011b), but it was presumed to be a non-host since the species is not a member of the Lauraceae. In the field, lychee bolts (from an unknown cultivar) were found to be highly attractive to *X. glabratus*, capturing significantly more beetles than host avocado wood or commercial manuka oil lures (Kendra et al. 2011). In no-choice laboratory bioassays conducted with the same lychee germplasm, more than half of *X. glabratus* females initiated boring when presented with lychee bolts.

Based on the latter observation, a study was initiated to evaluate host status of two commercial lychee cultivars, 'Brewster' and 'Mauritius', varieties planted extensively in south Florida (Crane et al. 2008). Since lychee is a subtropical fruit tree grown in close proximity to avocado groves in Miami-Dade County, lychee could potentially function as a reservoir for *R. lauricola* and/or *X. glabratus* even if it is not susceptible to laurel wilt. Several laboratory, greenhouse, and field experiments were conducted with 'Brewster' and 'Mauritius' to determine if these cultivars were susceptible to attack by *X. glabratus* and, if so, whether they develop symptoms of laurel wilt and support reproduction of *X. glabratus*. Plants were also artificially inoculated with *R. lauricola* to assess colonization of this host by the fungus and whether laurel wilt could be induced in this manner. In addition, we performed chemical analysis of the volatile emissions from lychee to determine how its sesquiterpene composition compares to that from known lauraceous hosts.

Materials and Methods

Laboratory Bioassays

Our initial experiment was a series of no-choice laboratory bioassays to assess host recognition and boring behaviors, following methods previously published (Kendra et al. 2011). Test substrates consisted of freshly-cut bolts (5 cm diam. × 15 cm length) from four trees: (1) *L. chinensis* cv. 'Brewster' [obtained from the USDA-ARS Germplasm Repository, Miami, Florida (WA3-24-61, MIA# 17454, PI# 277462)], (2) *L. chinensis* cv. 'Mauritius' [USDA-ARS (WA3-20-64, MIA# 17441, PI# 277473)], (3) silkbay *Persea humilis* Nash [a known host and positive control (Fraedrich et al. 2008), found to be the most attractive native *Persea* in previous field tests (Kendra et al. 2013)], and (4) live oak *Quercus virginiana* Mill. [Fagaceae, a non-host and negative control (Fraedrich et al. 2008; Mayfield et al. 2008; Kendra et al. 2011)]. Bolts of silkbay and live oak were collected from sites along the Lake Wales Ridge in central Florida (Highlands County). Test insects

were host-seeking female *X. glabratus* collected at those same field sites using a baiting method described previously (Kendra et al. 2012b). Insects were captured during the late afternoon/early evening, held overnight in plastic boxes containing moist tissue paper, and used in bioassays early the next morning. Assays were conducted at Archbold Biological Station (Lake Placid, Florida) under controlled laboratory conditions (25 °C, 16:8 h L:D). Test arenas consisted of 4.4-L plastic buckets covered with cheese cloth mesh, secured with rubber bands, and held in screened insect cages (BioQuip, Rancho Dominguez, California) during the experiment (Fig. 1A). In each bucket, lined with a filter paper disk (15 cm diam; Whatman Intl. Ltd., Maidstone, England), were placed 10-15 beetles and a single bolt of wood (Fig. 1B). A beetle was scored positive for boring when it was perpendicular to the wood substrate and half its body length (~1 mm) was inserted into the entrance hole (Fig. 1C). [Previous observations indicated that females sometimes 'sampled' the substrate (making shallow bore holes) but then aborted the attempt; however, once they inserted half their body length, they continued to bore through the bark and cambium layers into the sapwood.] The number of beetles that were boring and their location on the bolt were recorded at 1, 2, 4, 8, 12, 24, and 48 h. Tests were replicated 5 times for each substrate, and each replicate was run using a separate arena, a new bolt of wood, and a new cohort of beetles.

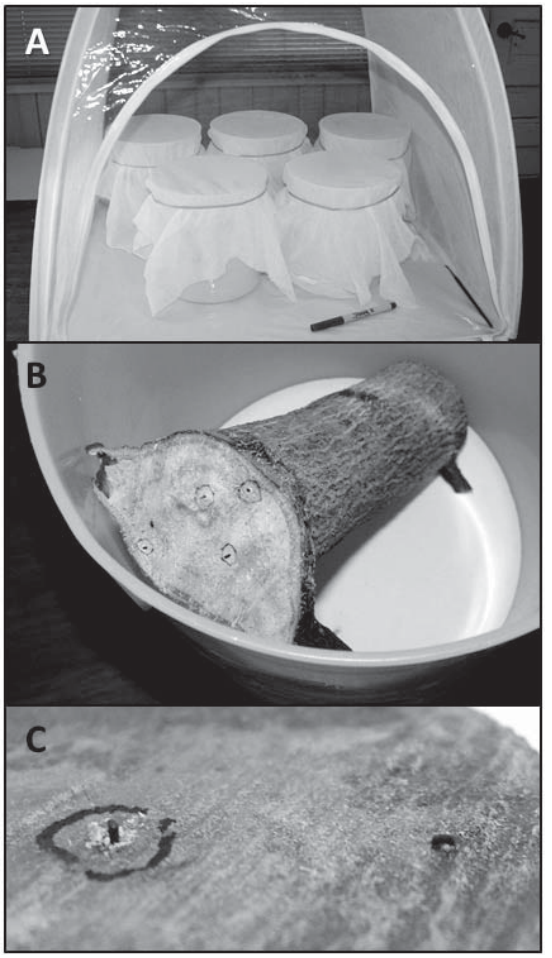

Fig. 1. Behavioral bioassays were conducted in 4.4 liter buckets covered with cheese cloth and held within screened insect cages (A). Each bucket contained 10-15 female *Xyleborus glabratus* and one bolt of wood (B). Bolts were checked at regular intervals and females were considered fully committed to boring (scored positive) when they were perpendicular to the substrate and half their body length (~1 mm) was inserted into the entrance hole (C, female on left). Note: the female on right has chosen a site and initiated boring (in head-down posture), but would not be scored positive in our bioassay.

Field Infestation Experiment

A second no-choice experiment was conducted to determine if *X. glabratus* would bore into live lychee trees and transmit *R. lauricola*, using protocols similar to those of Mayfield et al. (2008) for evaluation of avocado host status. Test substrates consisted of four potted 'Brewster' trees of two size classes, maintained outdoors under irrigation at the Lake Wales Ridge Environmental Management Area, Royce Ranch Unit, Highlands County, Florida (N 27° 22' 464", W 81° 20' 212"). Two small trees (Trees A and B), each 2.5 cm trunk diam at soil level, were purchased from a commercial nursery in Homestead, Florida. Two larger trees (Trees C and D), with trunk diam of 11.4 and 13.8 cm, were obtained from a local lychee grower by air-layering of branches from mature trees in his grove. With each of the four trees, one branch was sawed off (flush with the trunk) to provide a freshly-cut surface, since previous studies had shown a preference by *X. glabratus* to initiate boring at cut or wounded sites (Kendra et al. 2011). The portion of the trunk containing the cut surface was enclosed in a mesh insect sleeve (20 × 40 cm, BioQuip) which contained a zippered access port and a clear plastic window to allow for observations (Fig. 2A). Two thin metal hoops were placed inside the sleeve and secured with binder clips, which prevented the sleeve from collapsing and further facilitated observations. Each sleeved trunk then received 10 female *X. glabratus* (≤ 3 d post-emergence), obtained from the University of Florida laboratory colony, which had been reared under conditions described by Brar et al. (2013). The experiment was initiated in the early evening (18:00-18:30 h EDST), since this time period had been identified as the peak window during which female *X. glabratus* engage in host-seeking flight in Florida (Brar et al. 2012; Kendra et al. 2012a).

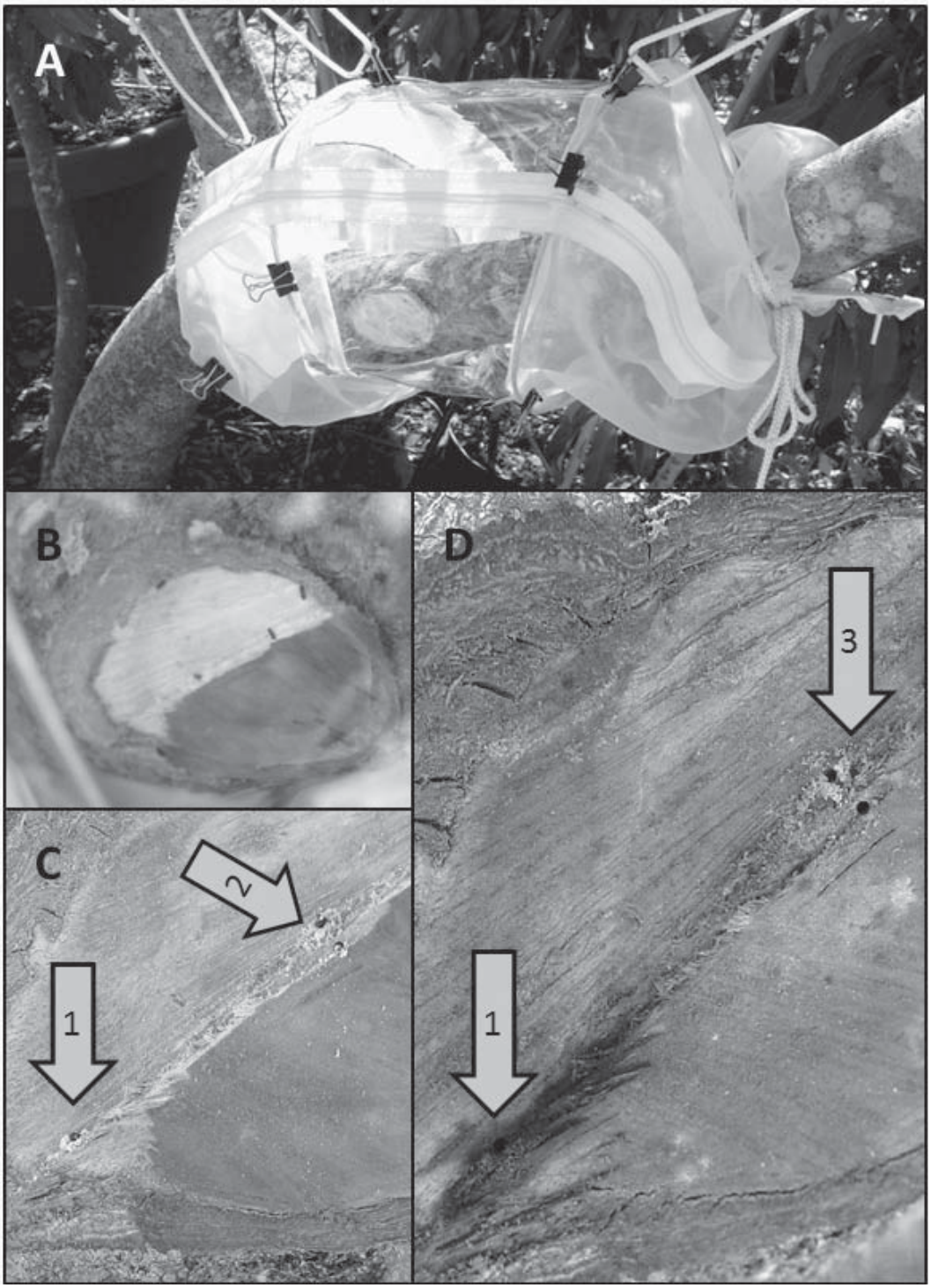

Fig. 2. Field infestation experiments were conducted with potted lychee trees (*Litchi chinensis* cv. 'Brewster'). One branch was pruned flush with the trunk, and this portion of the tree was then enclosed in a zippered mesh sleeve and 10 female *Xyleborus glabratus* were introduced (A). At 1 h, observations through the sleeve indicated that many beetles were walking over the freshly-cut surface (B). At 16 h, three beetles were actively boring into the cut surface (C). At 2 wk, four distinct entrance holes were observed on the tree, and fresh sawdust was present, indicative of active boring (D). (All photos of experimental Tree C.)

Initial observations (to confirm boring) were made after 1 h and 16 h (the next morning), and then the condition of the trees was checked thereafter at approximately 2-3 wk intervals for 3 mo. At the conclusion of the experiment, wood was dissected near the beetle entrance holes to examine gallery formation and search for evidence of *X. glabratus* reproduction.

In addition, tissue associated with five (Tree C) and six (Tree D) galleries of *X. glabratus* was assayed for *R. lauricola*. Brownish discoloration, often quite conspicuous, was observed adjacent to galleries when trunks were dissected lengthwise with a bandsaw (Fig. 3). With a hammer and chisel, discolored wood was sampled ca 0.5 cm from the above galleries, surface-disinfested for 15 sec in 70% ethanol and then 2 min in 10% bleach, rinsed in sterile water, and blotted dry with sterile Kimwipes before placement on a semi-selective medium, CSMA+, in 10-cm-diam Petri plates (Ploetz et al. 2012). Plates were incubated on a laboratory bench for 10 d under ambient light before examination for growth characteristic of *R. lauricola*.

Artificial Inoculation of Lychee with *Raffaelea lauricola*

RL4, an isolate of *R. lauricola* that kills lauraceous hosts of this pathogen (Ploetz et al. 2012; Ploetz & Konkol 2013), is deposited as CBS 127349 at the Centraalbureau voor Schimmelcultures (CBS Fungal Biodiversity Centre, Utrecht, The Netherlands); it was removed from -80 °C storage and grown on malt extract agar (MEA) for use in inoculation experiments. Trees of cvs. 'Brewster' and 'Mauritius' (air-layered plants in 12-L pots) were obtained from a local commercial nursery and maintained in an air-conditioned greenhouse (ca 28 °C) at the University of Florida's Tropical Research & Education Center in Homestead, Florida. Three trees of each cv were each inoculated ~20 cm above the soil line with 10^5 conidia of RL4 as described previously (Ploetz et al. 2012).

After 1 mo, trees were observed externally and internally for disease symptoms, including chlorotic and necrotic foliage, vascular discoloration, discharge from the inoculation sites, and any other indication of a pathological interaction of RL4 with inoculated trees. In addition, sapwood was sampled as above and assayed for *R. lauricola*. The experiment was conducted twice.

Chemical Collection and Analysis

Samples for chemical analysis were prepared by manually rasping the outer layers of bark and cambial tissue from freshly cut bolts (5 cm

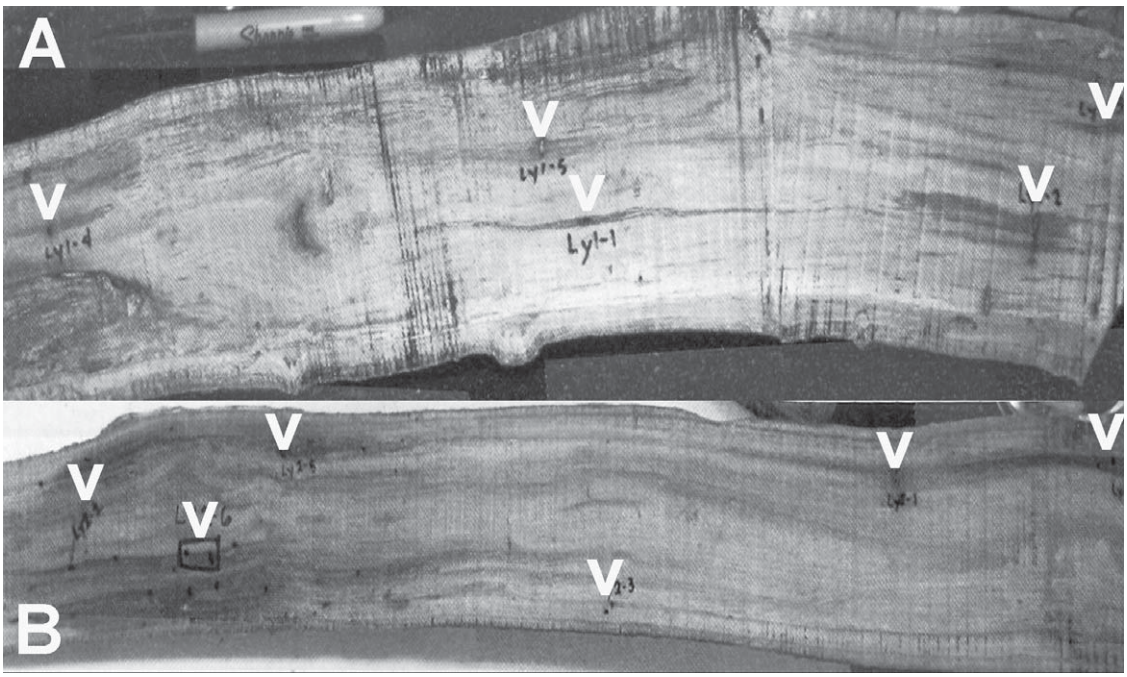

Fig. 3. Longitudinally dissected trunks from no-choice studies on potted lychee Trees C and D (*Litchi chinensis* cv. 'Brewster') in which galleries of *Xyleborus glabratus* have been exposed and indicated with "V's". Discolored tissue adjacent to the galleries was removed with a hammer and chisel and assayed for *Raffaelea lauricola*, as decribed in the text.

diam) of lychee cv. 'Brewster', silkbay, and live oak (identical to those used in bioassays above), using methods reported by Niogret et al. (2011a). Volatile chemicals were collected from the rasped shavings (6-g samples, 3 replicates per tree) by using Super Q traps (Analytical Research Systems, Gainesville, Florida) according to published methods (Heath & Manukian 1992; Heath et al. 1993). Samples were spread in a cylindrical glass chamber (4.5 cm diam × 25 cm length), 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 h 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, New Jersey). An aliquot of C_{16} standard (5 µg) was added to each sample for quantitative analysis. All chemical sampling was performed within 2 h of collection of the branch material.

Chemical extracts were analyzed by using gas-chromatography (ThermoQuest Trace GC 2000, Austin, Texas). The column was fused silica, 25 m long, 0.25 mm i.d. DB-5MS phase (J&W Scientific, Agilent Technologies, Santa Clara, California), 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 (NIST11) 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, 1532 for α-cubebene (Bedoukian Research Inc., Danbury, Connecticut), α-copaene (Fluka Analytical, Stenheim, Germany), β-caryophyllene (Sigma Chemical Co., St. Louis, Missouri), α-humulene (Sigma Chemical Co.), and δ-cadinene (Fluka Chemie, Buchs, Switzerland), respectively] or with previously published data [calamenene (Singh et al. 2007; Ibrahim et al. 2010)].

Statistical Analysis

Regression analysis was used to document temporal patterns in boring behaviors observed in the no-choice laboratory bioassays (Systat Software 2010). Final results for percentage of boring observed with the bolt treatments was analyzed by one-way analysis of variance (ANOVA), followed by least significant difference test (LSD) for mean separation, $P < 0.05$ (Systat Software 2010).

Results

Laboratory Bioassays

Composite results of the no-choice boring bioassays are presented in Fig. 4. Regression analysis with sigmoidal models best described the relationships between the time after presentation of lychee or silkbay bolts and the percentage of females boring [Silkbay: $y = 98.21 / (1 + e^{-[(x - 4.12) / 1.26]})$, $R^2 = 0.989$; lychee cv. 'Brewster': $y = 42.84 / (1 + e^{-[(x - 6.64) / 1.94]})$, $R^2 = 0.991$; and lychee cv. 'Mauritius': $y = 34.56 / (1 + e^{-[(x - 7.30) / 2.55]})$, $R^2 = 0.987$]. Boring was initiated quickly on bolts of silkbay, with the maximum percentage achieved within ~8 h. With both cultivars of lychee, females spent considerably more time walking over the wood substrate before choosing a site and committing to boring behaviors. This resulted in a large lag time (relative to silkbay) in boring response, with the maximum percentage reached at 12-15 h, and no further increase recorded at the 24 or 48 h readings. Few beetles responded to the bolts of live oak; the majority continued to wander throughout the arena during the course of the experiment, but several females were observed to settle into natural crevices or under the bark at the cut ends of the bolts. This behavior was interpreted to represent a thigmotactic response; it was observed to vary-

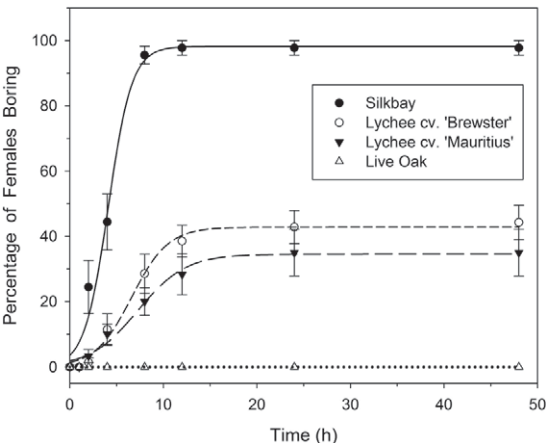

Fig. 4. Mean (± SE) percentage of female *Xyleborus glabratus* boring into wood bolts presented in a 48-h no-choice bioassay. Treatments consisted of silkbay (*Persea humilis*), lychee (*Litchi chinensis* cvs. 'Brewster' and 'Mauritius'), and live oak (*Quercus virginiana*). Rate of boring with silkbay and lychee was best fit by regression analysis with sigmoidal models (see text).

ing degrees with all bolt treatments and was not scored as positive for boring in our bioassays. At the conclusion of the test (48 h), there were differences in boring among the bolt treatments (F = 78.03, df = 3,16; $P < 0.001$). Percentage (mean ± SE) of females boring into silkbay (97.78 ± 2.22 %) was significantly higher than into 'Brewster' (44.29 ± 5.25 %) or 'Mauritius' (35.00 ± 7.17 %; not statistically different from 'Brewster'), and all three were higher than live oak (0%). Regarding site selection on the bolts, the percentage of boring initiated on the cut surface corresponded to 60.4% for silkbay, 85.7% for 'Mauritius', and 100% for 'Brewster'.

Field Infestation Experiment

One hour after introduction into the sleeved enclosures, the majority of female *X. glabratus* were walking over the freshly cut surfaces where branches had been removed from the four test trees (Fig. 2B), but no boring was observed at this time (following the same criteria for positive boring applied in the laboratory bioassays). When checked 16 h later (at 10:00 h the next morning), three beetles were actively boring into the cut surface on one of the large diam trees (Tree C, Fig. 2C), and four beetles were boring into the cut surface on the second large tree (Tree D). No boring was observed at any other site (i.e. through the bark) along the trunks of Trees C and D, and no boring was observed with either of the two small diam trees (Trees A and B). When checked again 2 wk later, Tree C had four distinct entry holes (representing 40% boring; Fig. 2D) and Tree D had five entry holes (50% boring), all located on the cut surface. Both trees also had fresh 'sawdust' present around the entrance holes which indicated that there was active boring by *X. glabratus*. In contrast, neither small tree had evidence of boring. At this 2-wk inspection, the sleeves were removed from all trees, and the dead 'founding' females (that had not bored) were removed from the enclosures. The sleeves were then reapplied to collect any F1 (first filial generation) adult *X. glabratus* that might emerge. Observations made over the next few months showed no indication of laurel wilt symptoms in either large diam tree into which *X. glabratus* had bored.

At 3 mo when the experiment was terminated, the sleeves were removed and no adult *X. glabratus* were found. Tree dissections revealed that the majority of beetle galleries were fairly shallow, consisting of an entrance tunnel with little or no branching into secondary tunnels, the sites of *X. glabratus* brood galleries (Brar et al. 2013). Although wood adjacent to galleries was discolored (Fig. 3), typical colonies of *R. lauricola* were not recovered on CSMA+ from tissue pieces associated with 11 galleries (0 of 102 assays).

Artificial Inoculation of Lychee with *Raffaelea lauricola*

After 1 mo, disease symptoms did not develop externally or internally in plants of either 'Brewster' or 'Mauritius' that were inoculated with *R. lauricola*; RL4 had no discernible impact on the health of inoculated trees during both experiments. Moreover, in no case was the fungus recovered from inoculated tissue.

Chemical Analysis

Table 1 presents the sesquiterpene emissions quantified from the bolt treatments used in the no-choice boring bioassays. Silkbay (which is highly aromatic) had the highest quantity of volatile sesquiterpenes as well as the largest diversity of sesquiterpene components. The

TABLE 1. QUANTITY (μG, MEAN ± S.D.) OF VOLATILE SESQUITERPENES EMITTED FROM WOOD BOLTS OF LYCHEE CV. 'BREWSTER' (*LITCHI CHINENSIS*), SILKBAY (*PERSEA HUMILIS*), AND LIVE OAK (*QUERCUS VIRGINIANA*). VOLATILES WERE ISOLATED FROM 6-G SAMPLES OF RASPED BARK/CAMBIUM BY SUPER Q COLLECTION, AND THEN ANALYZED BY GC-MS (DB-5MS COLUMN).

Peak	Chemical	RI[a]	Lychee cv. 'Brewster'	Silkbay	Live Oak
1	α-cubebene[b]	1361	0.1 ± 0.1	7.5 ± 4.4	0.1 ± 0.1
2	α-copaene[b]	1394	2.6 ± 3.2	12.3 ± 3.6	1.0 ± 0.3
3	Unknown 1	1404	0.0 ± 0.0	2.9 ± 1.5	1.3 ± 0.3
4	Unknown 2	1425	0.0 ± 0.0	6.3 ± 4.2	0.0 ± 0.0
5	β-caryophyllene[b]	1443	5.5 ± 6.4	25.4 ± 11.2	5.2 ± 0.3
6	α-humulene[b]	1478	1.1 ± 1.2	2.1 ± 1.0	0.3 ± 0.1
7	δ-cadinene[b]	1532	0.0 ± 0.0	1.5 ± 0.2	0.8 ± 0.3
8	calamenene[c]	1534	0.2 ± 0.2	4.8 ± 3.9	0.0 ± 0.0

[a]Mean Kovats Retention Index calculated from 3 replicates per species.
[b]Identification by NIST/EPA/NIH mass spectral library (NIST11), then verified by comparison with RI of synthetic chemicals (see text).
[c]Identification by NIST/EPA/NIH mass spectral library (NIST11), then verified by comparison with RI from published reports (see text).

five principal components (in descending order) emitted from silkbay (Fig. 5A) consisted of β-caryophyllene (peak 5), α-copaene (peak 2), α-cubebene (peak 1), an unidentified sesquiterpene (peak 4), and calamenene (peak 8). Lychee cv. 'Brewster' (Fig. 5B) had significantly fewer peaks detectable within the sesquiterpene range, but there were several key constituents found in common with silkbay, including β-caryophyllene, α-copaene, and α-humulene (peak 6); however, all were detected at much lower levels. Live oak (Fig. 5C) also emitted β-caryophyllene and α-copaene, but at levels lower than lychee.

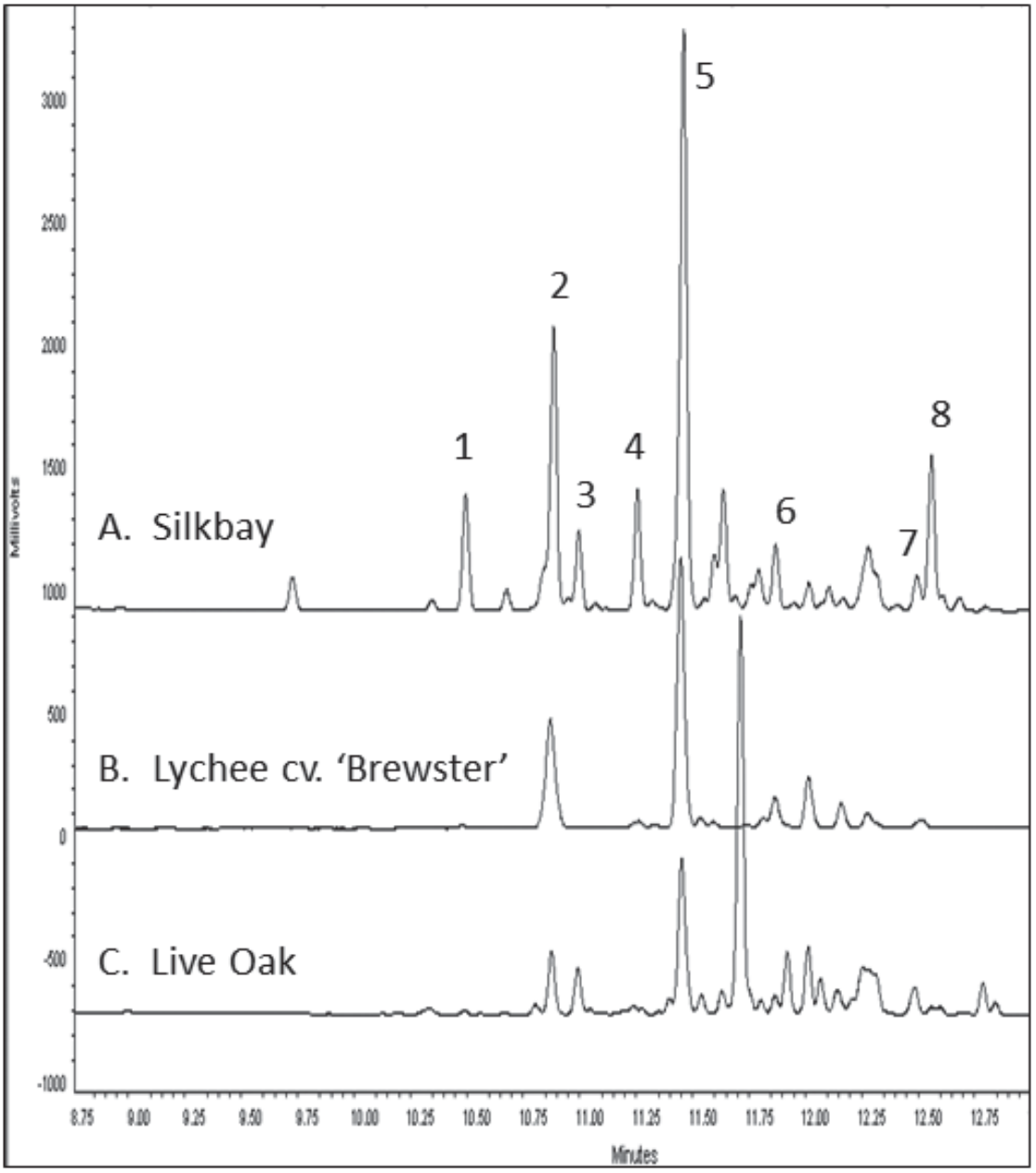

Fig. 5. Representative GC-MS analyses of sesquiterpenes obtained by Super Q collections from rasped bark/cambium of (A) silkbay (*Persea humilis*), (B) lychee (*Litchi chinensis* cv. 'Brewster'), and (C) live oak (*Quercus virginiana*). Peak identifications are as follows: 1 = α-cubebene, 2 = α-copaene, 3 = Unknown 1 (RI = 1404), 4 = Unknown 2 (RI = 1425), 5 = β-caryophyllene, 6 = α-humulene, 7 = δ-cadinene, and 8 = calamenene.

Discussion

In a previous study, *R. lauricola* was the most common symbiont recovered from *X. glabratus* (86% of assayed females with an average of 2,783 colony forming units per individual) (Carrillo et al. 2013). Although female *X. glabratus*, presumably carrying conidia of *R. lauricola* within mandibular mycangia (Fraedrich et al. 2008), successfully bored into lychee in the present no-choice tests, *R. lauricola* was neither recovered from areas adjacent to galleries of *X. glabratus*, nor were vascular symptoms (typical for laurel wilt) observed. Furthermore, RL4, an isolate of *R. lauricola* that kills lauraceous host plants (Ploetz et al. 2012, Ploetz & Konkol 2013), had no discernible impact on artificially inoculated lychee. The available data indicate that lychee cvs. 'Brewster' and 'Mauritius' are not hosts for *R. lauricola* and not susceptible to laurel wilt. Since lychee is native to Southeast Asia, it may have had a coevolved history with endemic *X. glabratus*, resulting in resistance to *R. lauricola* (a known symbiont of *X. glabratus* in Asia as well; Harrington et al. 2011). Further investigations would be necessary to determine if resistance is due to an induced defensive response by lychee trees or simply due to an unfavorable internal environment within lychee wood (e.g., moisture content, nutrient levels, secondary metabolites). Regardless, the inability of lychee wood to support growth of *R. lauricola*, the presumed primary nutritional symbiont of *X. glabratus*, also leads to the conclusion that *L. chinensis* is not likely to serve as a suitable reproductive host for *X. glabratus*. This is supported further by the lack of F1 adults 3 mo after gallery initiation into 'Brewster' lychee trees. In confirmed hosts, it has been shown that teneral adults are present within the galleries after 1 mo, and dispersing females are observed after 2 mo (Brar et al. 2013). However, the current study focused primarily on the threat of *X. glabratus* and *R. lauricola* to healthy, live trees of lychee (a specialty fruit crop of economic importance in south Florida); we cannot rule out the possibility that *X. glabratus* may potentially reproduce in stressed/dying lychee trees (from factors other than laurel wilt) which may have compromised resistance to ambrosia fungi. In lauraceous hosts, it has been hypothesized that optimal reproduction of *X. glabratus* is dependent on pre-colonization by *R. lauricola*; founding females may not necessarily lay eggs, but their inoculation of host trees with *R. lauricola* results in stress/disease conditions that facilitate successful reproduction of subsequent generations of *X. glabratus* (Fraedrich et al. 2008, 2011).

Although not a likely host for *X. glabratus*, lychee can be regarded as an effective 'chemical mimic' of the host Lauraceae. Lychee wood is attractive to dispersing, host-seeking females in the field (Kendra et al. 2011, 2012b), and therefore emits the appropriate long-range olfactory cues. Previous research with essential oils and lauraceous hosts suggests that α-copaene is the primary host attractant (kairomone), but other volatile sesquiterpenes may contribute; potential candidates include α-cubebene, α-humulene, β-caryophyllene, δ-cadinene, and calamenene (Hanula & Sullivan 2008; Kendra et al. 2011, 2012c, 2013; Niogret et al. 2011a, 2013a; Hanula et al. 2013). The chemical analysis of 'Brewster' in this study indicated that its sesquiterpene profile was much less complex than that of silkbay. 'Brewster's three main components – β-caryophyllene, α-copaene, and α-humulene – were also high in silkbay. Emissions of these same three sesquiterpenes were positively correlated with captures of *X. glabratus* in field tests with avocado bolts (three cultivars, Kendra et al. 2011), providing further support that these highly volatile terpenoids likely function as attractive kairomones.

In addition to attraction of *X. glabratus*, lychee wood also mimics a host in that it elicits boring behaviors from a significant proportion of females that make contact. This has been documented here with 'Brewster' (44%) and 'Mauritius' (35%), and previously with an unnamed cultivar (57%, Kendra et al. 2011). Although the lychee substrates may have been perceived as suboptimal, as evidenced by the longer assessment period (relative to silkbay) prior to boring observed in bioassays, lychee possesses sufficient secondary cues necessary for host recognition and acceptance by a subset of female *X. glabratus*. This phenomenon also has been observed with red maple *Acer rubrum* L. (Aceraceae), another non-host species. In no-choice tests with young potted plants, 40% of red maples were positive for *X. glabratus* boring, but those plants remained asymptomatic for laurel wilt, no *R. lauricola* were recovered, and no beetle reproduction was observed (Fraedrich et al. 2008). Little is known about the short-range secondary cues that trigger a behavioral switch from host-seeking to host-boring, but they may include olfactory, gustatory, and contact chemosensory cues in combination with tactile and visual stimuli (i.e., the wood substrate must smell, taste, feel, and look right in order for females to commit to boring). With either bolts or live trees of lychee, *X. glabratus* showed a strong boring preference for freshly-cut surfaces, presumably due to a higher release (threshold dose) of chemical stimulants from damaged tissue. In the bolt assay, significantly higher boring was obtained with silkbay, which emits much higher levels of sesquiterpenes than lychee. Therefore, the same chemicals responsible for long-range attraction may be associated with initiation of boring behaviors.

In the field infestation experiment, there was also evidence of a female preference for larger

diam hosts (boring percentages observed with potted 'Brewster' trees were 0, 40, and 50% with the 2.5, 11.4, and 13.8 cm diam trunks, respectively). Although anecdotal, the observation is consistent with data collected from infested forest ecosystems. In a survey of swampbay trees symptomatic for laurel wilt (N = 280), no *X. glabratus* entry holes (0.8 mm diam, Hanula et al. 2008) were observed in trunks/branches less than ~2 cm diam, and there was a progressive increase in density of entry holes with increasing diam (Kendra et al. 2013). The oldest (largest diam) trees are typically the first to be attacked by *X. glabratus* and succumb to laurel wilt; once those trees are depleted, the vector then attacks progressively smaller diam trees (Kendra et al. 2013). A host 'diameter preference' has also been observed in two rare Lauraceae, pondberry *Lindera melissifolia* (Walter) Blume and pondspice *Litsea aestivalis* (L.) Fern; although both species support growth of *R. lauricola* and are susceptible to laurel wilt when subjected to laboratory inoculations, the two shrubby plants with small diam stems are infrequently attacked by *X. glabratus* in the field, even when in close proximity to infested redbay trees (Fraedrich et al. 2011). This selective behavior for large woody hosts would be adaptive for *X. glabratus*, since larger diam trees could physically allow for larger gallery formations (and more extensive fungal gardens) within the sapwood, which would facilitate increased beetle reproduction. A positive correlation between gallery length and number of progeny has been demonstrated previously for other ambrosia beetles, including *Xylosandrus mutilatus* Blandford (Karimura & Hijii 1994) and *Xyleborus pfeili* (Mizuno & Kajimura 2002). Recent research indicates that host diam is a visual host-seeking cue used by female *X. glabratus* (Mayfield & Brownie 2013), but most likely effective when accompanied by appropriate chemical cues. Proximo-distal gradients (from trunk to large diam branches to small diam braches) in terpenoids, including α-copaene, have been documented within avocado trees (Niogret et al. 2013a, 2013b); these chemical gradients, in combination with visual cues, may function in the discrimination process used by *X. glabratus* for site-selection on host trees.

In summary, our results indicate that lychee may be vulnerable to attack by *X. glabratus*, particularly at sites with recent damage (e.g. freshly pruned limbs), due to chemical similarities with the Lauraceae. However, lychee cvs. 'Brewster' and 'Mauritius' are not susceptible to laurel wilt disease and do not promote growth of *R. lauricola.* Consequently, these commercial cultivars are not likely to function as reproductive hosts for *X. glabratus*. The discovery that lychee chemically mimics the host Lauraceae, but has a much simpler volatile profile, may provide a serendipitous opportunity to better understand the chemical ecology of *X. glabratus*. Further evaluations of chemically distinct lychee cultivars are underway to provide confirmation of the specific kairomones utilized by female *X. glabratus* for host location and recognition.

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