Comparative Analysis of Terpenoid Emissions from Florida Host Trees of the Redbay Ambrosia Beetle, Xyleborus glabratus (Coleoptera: Curculionidae: Scolytinae)

Authors: Niogret, Jerome, Kendra, Paul E., Epsky, Nancy D., and Heath, Robert R.

Source: Florida Entomologist, 94(4) : 1010-1017

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.094.0439

COMPARATIVE ANALYSIS OF TERPENOID EMISSIONS FROM FLORIDA HOST TREES OF THE REDBAY AMBROSIA BEETLE, *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

JEROME NIOGRET, PAUL E. KENDRA*, NANCY D. EPSKY AND ROBERT R. HEATH
United States Department of Agriculture, Agricultural Research Service,
Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158-1857 USA

*Corresponding author; E-mail: paul.kendra@ars.usda.gov

ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring insect that vectors *Raffaelea lauricola,* the fungal pathogen responsible for laurel wilt, a lethal disease of trees in the Lauraceae. First detected in the U.S. near Savannah, GA in 2002, *X. glabratus* has since spread throughout the southeastern coastal plain causing high mortality in native *Persea* species, particularly redbay (*P. borbonia*) and swampbay (*P. palustris*). Currently, breeding populations of *X. glabratus* pose an imminent threat to the avocado (*P. americana*) industry in south Florida. There is a critical need for effective attractants to detect, monitor, and control the spread of this invasive pest. In an effort to identify host-based attractants for dispersing female *X. glabratus*, we conducted a comparative study of the volatile chemicals emitted from wood of six species of Lauraceae found in Florida: avocado (*Persea americana*), redbay (*P. borbonia*), swampbay (*P. palustris*), silkbay (*P. humilis*), camphor tree (*Cinnamomum camphora*), and lancewood (*Ocotea coriacea*). We compared chemical profiles to those obtained from wood of lychee, *Litchi chinensis* (Sapindaceae), a presumed non-host found to be highly attractive to *X. glabratus* in field tests. GC-MS analysis identified 11 terpenoid compounds common to all lauraceous species. Of these, 4 sesquiterpenes were also found in lychee: a-copaene, b-caryophyllene, a-humulene and cadinene. Future research will include field tests and laboratory bioassays to evaluate the roles of each of these potential kairomones.

Key Words: Lauraceae, avocado, sesquiterpenes, kairomones, a-copaene, b-caryophyllene, a-humulene and cadinene

RESUMEN

El escarabajo ambrosia de laurel rojo, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), es un insecto barrenador exótico que es un vector de *Raffaelea lauricola*, el hongo patógeno responsable de la marchitez del laurel, una enfermedad mortal de los árboles de la familia Lauraceae. Detectado por primera vez en los EE.UU. cerca de Savannah, Georgia en el año 2002, *X. glabratus* se ha extendido su rango desde entonces a la llanura costera del sureste causando mortalidad alta en las especies nativas de *Persea*, en particular del laurel rojo (*P. borbonia*) y swampbay (*P. palustris*). En la actualidad, las poblaciones reproductoras de *X. glabratus* representan una amenaza inminente para la industria del aguacate (*P. americana*) en el sur de la Florida. Hay una necesidad critica de atrayentes eficaces para detectar, monitorear y controlar la propagación de esta plaga invasora. En un esfuerzo por identificar atrayentes con base a los hospederos para la dispersión de hembras de *X. glabratus*, se realizó un estudio comparativo de los productos químicos volátiles emitidos a partir de madera de seis especies de Lauraceae en Florida: aguacate (*Persea americana*), del laurel rojo (*P. borbonia*), swampbay (*P. palustris*), silkbay (*P. humilis*), árbol de alcanfor (C*innamomum camphora*), y lancewood (*Ocotea coriacea*). Se compararon los perfiles químicos con los perfiles obtenidos de madera de lichi, *Litchi chinensis* (Sapindaceae), que supuestamente no es un hospedero de *X. glabratus* pero fue muy atractiva para S. glabratus en pruebas de campo. El análisis de CG-EM (Cromatografia de Gases y Espectrometria de Masas) identificó nueve compuestos terpenoides común a todas las especies de Lauraceae. De ellos, tres sesquiterpenos se encontraron también en lichi: a-copaeno, b-cariofileno, a-humuleno y cadineno. Las investigaciones futuras se incluiran pruebas

Ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) comprise a diverse group of morphologically-similar wood borers that cultivate and feed on symbiotic fungi growing on the xylem of host trees (Rabaglia 2002). Typically infesting weak or dying trees, most species are not of economic importance and have not been studied well. The redbay ambrosia beetle, *Xyleborus* *glabratus* Eichhoff, is not a pest in its native distribution in southeastern Asia. It acquired pest status upon entry into the U.S., where it vectors the fungal pathogen, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Harrington et al. 2008), that causes laurel wilt, a newly described lethal disease of trees in the family Lauraceae (Fraedrich et al. 2008). First detected in 2002 near Savannah, Georgia (Rabaglia et al. 2006), *X. glabratus* has since spread into the Carolinas, Florida and Mississippi (USDA-FS 2011). *Xyleborus glabratus* functions as a primary colonizer of U.S. hosts, capable of attacking apparently healthy trees (Hanula et al. 2008). During gallery excavation, females introduce spores of several symbiotic fungal species (Harrington et al. 2010). While providing food for the adults and larvae, the symbiotic fungus also invades the host vascular system. Infected trees respond to *R. lauricola* by secreting resins and forming walls (parenchymal tyloses) within the xylem vessels. This defensive response results in blockage of water transport, systemic wilt, and ultimately tree death, which has been observed to occur in as little as 6 weeks under experimental conditions (Mayfield et al. 2008).

Native *Persea* species in north and central Florida have suffered high mortality due to laurel wilt, particularly redbay [*P. borbonia* (L.) Spreng.] and swampbay [*P. palustris* (Raf.) Sarg.], which appear to be preferred hosts in the U.S. Fraedrich et al. (2008) reported that redbay mortality increased from 10% to more than 90% in 15 mo at the site where *X. glabratus* was first detected in Florida. Redbay plays an important role in the natural ecosystem, furnishing food (fruit, seed and foliage) for songbirds, wild turkey, quail, deer, and black bear (Brendemuehl 1990; Fraedrich et al. 2007), and *Persea* species are the primary larval hosts of the palamedes swallowtail, *Papilio palamedes* Drury, Lepidoptera: Papilionidae (Scott 1986). Other Lauraceae susceptible to laurel wilt disease include silkbay (*Persea humilis* Nash), 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] of which the latter two are listed as endangered species at the state and/or federal level (Fraedrich et al. 2008; HSPD-9 2009). The widespread availability of host trees in Florida, in combination with human transport of infested material (primarily firewood, FDACS-DPI 2010), have contributed to a rapid southward spread of the vector-disease complex, which currently threatens another *Persea* species, avocado (*P. americana* Mill). In February 2011, laurel wilt and breeding populations of *X. glabratus* were detected in stands of swampbay trees in Miami-Dade County (FDACS 2011), less than 9 miles (14.4 km) north of commercial avocado groves. Avocado production represents an estimated $14 million annually at the farm gate for the state of Florida (USDA-NASS 2011). In addition, replacement costs of commercial and residential avocado trees in Miami-Dade, Broward, Palm Beach and Lee Counties have been estimated at $429 million (Evans & Crane 2008).

Due to the serious ecological and economic impact of laurel wilt disease, there is a critical need for effective attractants to detect, monitor, and control the spread of the vector. To date, no aggregation pheromone has been identified for *X. glabratus*. Initial research by Hanula et al. (2008) indicated no significant attraction of *X. glabratus* to its fungal symbiont, to its frass, or to ethanol (the standard attractant for ambrosia beetles). Host tree volatiles (secondary metabolites) appear to be the primary attractants used by dispersing females. Additional studies conducted in South Carolina (Hanula & Sullivan 2008) identified 2 essential oils as attractive baits for monitoring *X. glabratus*, i.e., manuka oil and phoebe oil [essential oil extracts from the tea tree, *Leptospermum scoparium* Forst. & Forst. (Myrtaceae) and the Brazilian walnut, *Phoebe porosa* Mex. (Lauraceae), respectively]. Based on that report, the current monitoring system for *X. glabratus* consists of Lindgren multi-funnel traps baited with manuka oil lures (CISEH 2010). However, recent field tests conducted in Florida indicated that manuka lures were not competitive with host avocado wood, had limited longevity (Kendra et al. 2011a), and captured numerous non-target ambrosia beetles (Kendra et al. 2011b). Phoebe lures were found to be highly effective for capture of *X. glabratus* (Kendra et al. 2011a), but unfortunately, they are no longer available due to depletion of source trees in the Brazilian rain forest. Based on a comparison of volatile emissions from manuka oil, phoebe oil, and chipped redbay wood, Hanula & Sullivan (2008) hypothesized that two sesquiterpenes, a-copaene and calamenene, were host-based attractants (kairomones) for *X. glabratus*.

Terpenoids (C_{10} monoterpenes and C_{15} sesquiterpenes) are secondary plant metabolites known to play various roles in insect-host interactions. They are located within the cambium layers (defined as the lateral meristem including the vascular cambium and cork cambium in vascular plants), and typically consist of several major compounds accompanied by derivatives and minor components. Some terpenoids function as defensive compounds, being either toxic or repellent to deter attacks by phytophagous insects (Gershenzon & Croteau 1991; Byers et al. 2000; Byers et al. 2004). Terpenoids emitted from herbivore-damaged plants have been shown to attract natural enemies and confer indirect plant protection (Pettersson et al. 2000). Terpenoids also function prominently in host location by herbivorous insects (Miller & Strickler 1984; Visser 1986). Previous studies showed that bark beetles utilize terpene olfactory cues for host recognition, finding appropriate host trees either by attraction to, or repulsion from, plant volatiles from a distance (Byers 1995).

This report presents results from a comparative study of volatile chemicals emitted from known host trees of *X. glabratus*. Using gas chromatography-mass spectroscopy (GC-MS) of volatiles collected from wood substrates, we identified and compared the principal terpenoids emitted from avocado and five species of Lauraceae that occur sympatrically in south Florida, i.e., redbay, swampbay, silkbay, camphor tree, and lancewood [*Ocotea coriacea* (Sw.) Britton]. Lancewood has not been confirmed a host for *X. glabratus*, but was included in this study as a potential host since it is a laurel tree native to south Florida, commonly found in coastal hammocks and pinelands. We compare the chemical profiles from the Lauraceae to that obtained from wood of lychee (*Litchi chinensis* Sonn.; Sapindaceae), a presumed non-host recently found to be high in a-copaene (Niogret et al. 2011) and highly attractive to *X. glabratus* in field tests (Kendra et al. 2011a). We also evaluate results of the chemical analyses in terms of attraction and behavioral responses of *X. glabratus* documented in previous laboratory and field studies. With this comprehensive approach, we hope to gain a better understanding of the chemical ecology of this new invasive species, and more specifically to identify chemical attractants with potential for improved pest detection and control.

MATERIALS AND METHODS

Evaluation of Sampling Method

Preliminary chemical analyses were performed to determine if sample preparation by manual rasping of avocado tree branches (Niogret et al. 2011) resulted in the induction of volatile chemicals not present in undamaged branches. Samples were collected outdoors from the branches of 3 field-grown avocado trees (8 cm branch diam., 3 replicate samples per tree). Volatile collections were performed using an outdoor super-Q collection system consisting of an oven bag (Reynolds, Richmond, Virginia) tightly wrapped around: 1) a 30 cm length of intact branch, and 2) a 30 cm length of the same branch immediately after manual rasping of bark and cambium (5 cm^2 area). The bag was connected to push-pull pumps (Cole-Parmer, Vernon Hills, Illinois) producing a 1 L min^{-1} air flow, powered by a 12 volt car battery. Volatile chemicals were collected from the intact and damaged branches using traps containing super-Q as the adsorbent (Analytical Research Systems, Inc., Gainesville, Florida) connected to the bag for 60 and 30 min, respectively. Chemicals were eluted from the super-Q adsorbent using 200 µL of high purity methylene chloride (99.5% pure; ACROS, Morris Plains, New Jersey). Chemical profiles from intact and damaged branches were qualitatively compared with the chemical profiles from the rasped cambial samples (1g) prepared using the protocol described below.

Host Material

Seven tree species were evaluated in this study, consisting of avocado (cv 'Brooks Late', *P. americana* var. *guatemalensis* Williams) and lychee (cv 'Hanging Green') obtained from the germplasm collection at the USDA-ARS Subtropical Horticulture Research Station (Miami, Florida); silkbay collected from the Lake Wales Ridge at Archbold Biological Station (Lake Placid, Florida); redbay collected at Snyder Park (Ft. Lauderdale, Florida); swampbay collected at Deering Estate (Miami, Florida); camphor tree collected at Montgomery Botanical Center (Miami, Florida), Bok Tower Gardens (Lake Wales, Florida), and a private residence (Lake Placid, Florida); and lancewood collected at Matheson Hammock (Miami, Florida).

Chemical Collection and Analysis

Samples for chemical analysis of host trees were prepared by manually rasping the outer layers (bark and cambial tissue) from freshly-cut branches (4.4 ± 0.6 cm diam., 3-9 replicate trees per species) using a microplaner. Samples were collected and analyzed within 24 h after branch removal, and within 5 min after rasping the cambium. Volatiles were collected from rasped wood samples (6 g per replicate) using traps filled with super-Q as the adsorbent (Analytical Research Systems) according to methods published previously (Heath & Manukian 1992; Heath et al. 1993). Wood 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^{-1}), and headspace volatiles were collected for 15 min. Collector traps were cleaned by soxhlet extraction using methylene chloride for 24 h and dried in a fume hood prior to use. Volatile chemicals were eluted from the super-Q adsorbent using 200 µL of high purity methylene chloride (99.5% pure; ACROS). For quantitative analysis, an aliquot of C_{16} standard (5 µg) was added to each sample prior to injection onto the GC column.

Chemical extracts were analyzed using a gas-chromatograph (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 50-130 °C at 15.0 °C min^{-1}, then from 130-220 °C at 10.0 °C min^{-1} and held at 220 °C for 4 min. The following parameters were used for the Mass Spectrometer (Agilent Technologies 5975B): EI energy: 69.9eV, MS source and MS quadrupole at 230 and 150 °C respectively, Electron Multiplier 1294V. The column used in the gas chromatograph interface to the mass spectrometer 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 40-130 °C at 10.0 °C min^{-1}, then from 130-220 °C at 20.0 °C min^{-1} and then held at 220 °C for 4 min. Chemicals were identified using the NIST Mass spectral program version 2.0d and NIST/EPA/NIH mass spectral library (NIST05) when Reverse Matches and Matches were >950 and >900‰, respectively.

For each terpenoid (Table 1), the Kovats Retention Index (RI) was calculated based on separation on the DB-5MS column. To verify chemical identifications generated by the mass spectral library, the RI values of sample peaks were compared with the RI values obtained from synthetic chemicals (when commercially available) or with previously published data obtained with comparable GC methods. Synthetic chemicals consisted of a-pinene (Aldrich Chemical Co., Milwaukee, Wisconsin), b-pinene (Aldrich Chemical Co.), limonene (Glidden Organics of SCM Corp., Jacksonville, Florida), a-cubebene (Bedoukian Research, Inc., Danbury Connecticut), a-copaene (Fluka Analytical, Stenheim, Germany), b-caryophyllene (Sigma Chemical Co., St. Louis, Missouri), a-humulene (Sigma Chemical Co.) and cadinene (Fluka Chemie, Buchs, Switzerland). We were not able to confirm the identification of chemicals emitted in very low quantity (<1µg) but we compared their RI with RI from published reports (a-thujene (Singh et al. 2007) and calamenene (Ibrahim et al. 2010)). Calamenene and cadinene were previously reported in the literature in redbay (Hanula & Sullivan 2008) and b-caryophyllene was previously reported in seeds and leaves of camphor trees (Yong & Wenyi 2002; Zhu & Guo 2010).

Results and Discussion

Evaluation of Sampling Method

No qualitative differences in chemical profiles were detected among the 3 avocado samples (intact branch, newly-damaged branch, and newly-rasped cambial sample), leading to the conclusion that production of chemicals was not induced at detectable levels during the time period evaluated (30 min). Newly-rasped samples contained the same chemical components as the intact branches, only at much higher levels, which resulted in improved sensitivity in detection of host secondary metabolites. Research with the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), showed increased attraction of beetles to stressed (girdled) host trees (Crook et al. 2008). That attraction was correlated with increased production of several sesquiterpenes, however elevated levels of those induced compounds were not observed until 24 h after tree injury.

Chemical Analysis

A total of 55 volatile chemicals (detected at quantities ≥ 0.5 µg in at least one of the replicate samples) were isolated and identified by super-Q collections and GC-MS analysis. Of those compounds, there were 11 terpenoids (4 monoterpenes and 7 sesquiterpenes) common to the lauraceous hosts, which can be regarded as constituting a generalized bouquet or 'volatile signature' of the Lauraceae (Table 1). Among the species evaluated, the terpenoid profile varied both qualitatively and quantitatively. Two of those compounds (RI 1047 and 1425) have yet to be identified due to inconsistent matches in the NIST mass spectral library.

Monoterpenes, particularly a- and b-pinene, were the dominant volatiles detected from the native *Persea* species. b-pinene made up 51, 45, and 21% of the terpenoid emissions from silkbay, redbay, and swampbay samples, respectively; and a-pinene was the dominant compound (41%) in swampbay. Camphor tree also had high monoterpene emissions, consisting of 12, 12, and 10% of a-thujene, b-pinene and an unknown monoterpene (RI 1047), respectively. The lancewood profile was composed almost entirely of pinene volatiles (58 and 37% b- and a-pinene, respectively). Pinenes have been documented as attractants for related species of ambrosia beetles, including *Xyleborus pubescens* Zimmermann (Miller & Rabaglia 2009) and *Xyleborinus saxesenii* (Ratzeburg) (Petrice et al. 2004). However, pinene emissions from avocado, a confirmed host (Mayfield et al. 2008), were much lower than from the 3 native *Persea* spp., and only trace amounts of pinenes (and monoterpenes in general) were detectable from lychee wood, a substrate highly attractive to *X. glabratus* and other Scolytinae in Florida (Kendra et al. 2011a,b). These data suggest that, although pinenes are released at high levels from the preferred hosts (native *Persea* spp.), they are not primary host attractants for female *X. glabratus*. Hanula et al. (2008) came to the same conclusion, finding no attraction of *X. glabratus* to freshly-cut bolts of loblolly pine (*Pinus taeda* L.), a species high in both a- and b-pinene. It is plausible, however, that pinenes may function as synergistic attractants when they co-occur with appropriate laurel sesquiterpenes, such as a-copaene. Synergism among volatile attractants has been well

TABLE 1. QUANTITY (µG, MEAN ± SD) OF VOLATILE TERPENOIDS EMITTED FROM 6 G RASPED WOOD SAMPLES FROM AVOCADO (*PERSEA AMERICANA*), SILKBAY (*PERSEA HUMILIS*), SWAMPBAY (*PERSEA PALUSTRIS*), REDBAY (*PERSEA BORBONIA*), CAMPHOR TREE (*CINNAMOMUM CAMPHORA*), LANCEWOOD (*OCOTEA CORIACEA*), AND LYCHEE (*LITCHI CHINENSIS*). VOLATILES WERE ISOLATED BY SUPER-Q COLLECTION AND GC-MS ANALYSIS (DB-5MS COLUMN).

Peak	Chemical	RI[a]	Avocado	Silkbay	Swampbay	Redbay	Camphor	Lancewood	Lychee
1	a-thujene	932	0.6 ± 0.1[d]	0.3 ± 0.2[d]	0.6 ± 0.4[d]	0.3 ± 0.2[d]	9.0 ± 3.2[d]	<0.05[e]	<0.05[e]
2	a-pinene	944	7.8 ± 1.3[c]	24.0 ± 27.7[c]	44.5 ± 26.8[c]	11.9 ± 9.2[c]	3.4 ± 1.9[c]	19.5 ± 17.4[c]	0.3 ± 0.3[c]
3	b-pinene	992	6.6 ± 1.1[c]	100.3 ± 60.3[c]	22.0 ± 12.4[c]	49.0 ± 17.4[c]	8.6 ± 4.4[c]	30.3 ± 19.5[c]	<0.05[c]
4	Unknown 1	1047	3.9 ± 1.2	18.9 ± 19.3	17.9 ± 19.9	28.1 ± 43.4	7.6 ± 7.9	<0.05	<0.05
5	a-cubebene	1360[c]	11.4 ± 1.8[c]	7.5 ± 4.4[c]	5.1 ± 5.1[c]	1.9 ± 0.7[bd]	9.6 ± 4.2[c]	0.2 ± 0.2[c]	0.4 ± 0.0[c]
6	a-copaene	1394	12.9 ± 5.0[c]	12.3 ± 3.6[c]	8.9 ± 2.9[c]	10.3 ± 3.4[c]	23.0 ± 12.3[c]	1.6 ± 0.7[c]	23.6 ± 3.5[c]
7	Unknown 2	1425	0.3 ± 0.1	6.3 ± 4.2	7.7 ± 6.8	7.0 ± 2.1	0.4 ± 0.2	0.1 ± 0.0	0.3 ± 0.3
8	b-caryophyllene	1442	14.4 ± 7.6[c]	25.4 ± 11.2[c]	<0.05[e]	0.9 ± 0.2[c]	10.4 ± 6.7[b]	0.2 ± 0.10[c]	15.8 ± 4.7[c]
9	a-humulene	1477	1.3 ± 0.7[c]	2.1 ± 1.0[c]	0.9 ± 1.3[e]	0.2 ± 0.1[e]	0.6 ± 0.2[e]	0.1 ± 0.1[e]	3.6 ± 0.5[c]
10	Cadinene	1532	1.6 ± 0.7[c]	1.5 ± 0.2[c]	0.9 ± 1.0[d]	0.6 ± 0.2[bd]	2.4 ± 0.7[c]	0.3 ± 0.3[c]	2.3 ± 0.5[c]
11	Calamenene	1538	1.3 ± 1.2[d]	4.8 ± 3.9[d]	4.5 ± 4.6[d]	1.4 ± 1.6[bd]	1.1 ± 0.4[e]	0.1 ± 0.0[d]	0.1 ± 0.1[d]

[a]Mean Kovats Retention Index calculated from 3-9 replicates per species.
[b]Previously reported in published reports (see text).
[c]Identification verified by comparison with synthetic chemicals (see text).
[d]Identification by NIST library.
[e]Identification verified by comparison with RI from published reports (see text).

documented in other insect systems (Heath et al. 2004; Kendra et al. 2008).

Among the sesquiterpenes detected, there were 7 chemicals found in common to the 6 species of Lauraceae (Fig. 1, peaks 5-11). Of those 7, 4 were also present as significant components in the lychee sample (Fig. 1G): a-copaene (peak 6), b-caryophyllene (peak 8), a-humulene (synonymous with b-caryophyllene; peak 9; this chemical was identified in avocado and silkbay but not clearly confirmed in other samples due to the low amount emitted, despite a similar RI) and cadinene (peak 10; analysis could not discern enantiomeric configuration). As 'common denominators' among the attractive wood substrates, these 4 sesquiterpenes are the most likely candidates that should be evaluated as kairomones for *X. glabratus*. Each of these sesquiterpenes has been implicated previously as a host-based semiochemical for other species of wood-boring beetle. For example, a-copaene, b-caryophylleneand a-humulene (emitted at elevated levels from stressed ash trees), all elicit consistent olfactory responses in electroantennographic analyses with *A. planipennis* (Crook et al. 2008); and a- and b-cadinene are known attractants for the European elm bark beetle, *Scolytus multistriatus* (Marsham), a vector of Dutch elm disease (Millar et al. 1986).

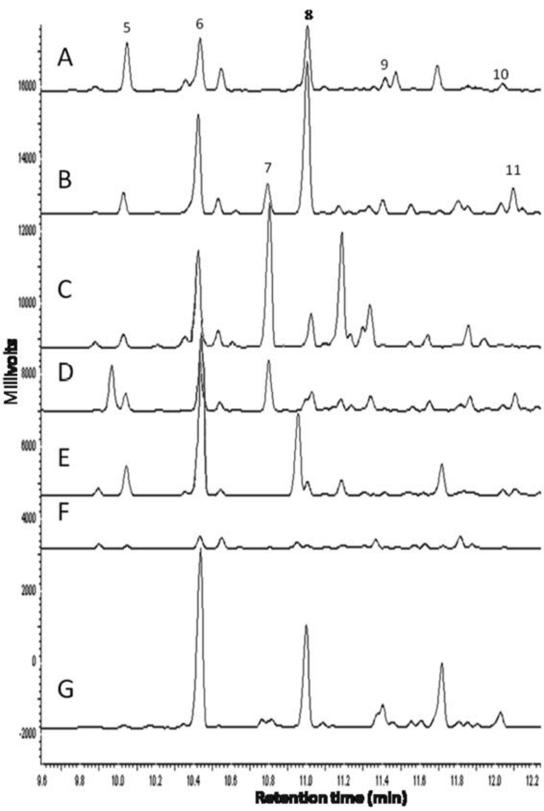

Fig. 1. Representative GC analyses (DB-5MS column) of sesquiterpenes obtained by super-Q collections from wood of (A) avocado (*Persea americana*), (B) silkbay (*Persea humilis*), (C) redbay (*Persea borbonia*), (D) swampbay (*Persea palustris*), (E) camphor tree (*Cinnamomum camphora*), (F) lancewood (*Ocotea coriacea*), and (G) lychee (*Litchi chinensis*). Peak numbers correspond to chemical identifications presented in Table 1.

The results of this comparative host study are consistent with results from previous field tests (Kendra et al. 2011a) that evaluated attraction of *X. glabratus* to manuka lures, phoebe lures, lychee wood (cultivar unknown), and avocado wood (3 cultivars representative of West Indian, Guatemalan, and Mexican races). In that study, field captures of *X. glabratus* were positively correlated with substrate levels of a-copaene, b-caryophyllene and a-humulene. Lychee wood had the highest a-copaene content and also caught significantly more *X. glabratus* than any of the other treatments. Lychee wood was also more attractive than avocado wood in two-choice laboratory bioassays (Kendra et al. 2011a). In the current study, a-copaene made up 55% of the total emissions from lychee (cv 'Hanging Green'), and was a prominent sesquiterpene in all of the lauraceous hosts, especially the *Persea* species. These results support the conclusion of Hanula and Sullivan (2008) that a-copaene functions as a primary host attractant for *X. glabratus*.

Hanula and Sullivan (2008) also identified calamenene as another potential kairomone based on a comparison of volatiles from redbay wood, manuka oil, and phoebe oil (both essential oils in neat form). However, our GC analyses detected calamenene (Fig. 1, peak 11), as a major peak only in wood from silkbay and swampbay but this chemical was not clearly identified in camphor. Moreover captures of *X. glabratus* were not correlated with calamenene in previous field tests with avocado and lychee wood (Kendra et al. 2011a). In addition, quantification of calamenene was low from commercial phoebe lures after just 1 week in the field (the dominant volatile emitted was a-copaene, 38%), despite high captures of *X. glabratus* over the 8-wk test (Kendra et al. 2011a). Therefore, calamenene did not appear to play a significant role in attraction to avocado, lychee, or phoebe oil lures, but it may potentially be a synergistic attractant in the native *Persea* species. Neither calamenene nor a-copaene has been evaluated directly as a field lure, due to lack of commercial availability in quantities sufficient for field tests.

As a side note, there is current disagreement among plant taxonomists regarding species and varietal differences among the *Persea* representatives in the southeastern U.S. (Brown & Kirkman 1990; Coder 2006). The chemical analyses presented herein identified both qualitative and quantitative differences among monoterpenes and sesquiterpenes from freshly-rasped wood of redbay, swampbay, and silkbay trees. These chemotaxonomic data, in combination with traditional morphological characters, may facilitate a better understanding of the phylogenetic relationships within the genus *Persea*.

Conclusions

A comprehensive comparison of volatile chemicals emitted from host trees in the Lauraceae and other attractive substrates (lychee wood, manuka oil, phoebe oil) identified 4 sesquiterpenes as potential host-based attractants for *Xyleborus glabratus*: a-copaene, b-caryophyllene, a-humulene and cadinene. Multiple lines of evidence suggest that a-copaene is a primary kairomone, requisite for attraction of dispersing females. Other sesquiterpenes (and monoterpenes) may potentially synergize attraction and/or be necessary for host recognition. Future research will include field tests and laboratory bioassays to assess relative attraction of *X. glabratus* to the Lauraceae of south Florida, and electroantennography will be explored to evaluate chemoreceptive response to candidate semiochemicals.

Acknowledgments

The authors thank Ricardo Joseph, Wayne Montgomery, and Mike Winterstein (USDA-ARS, Miami, Florida) for technical assistance; Patti Anderson (FDACS-DPI; Gainesville, Florida) for *Persea* identifications; Gretchen Pruett, Fred Lohrer, and Carl Weekley (Archbold Biological Station; Lake Placid, Florida), Tracy Magellen (Montgomery Botanical Center; Miami, Florida), Katrina Noland and Cheryl Peterson (Bok Tower Gardens; Lake Wales, Florida), Karolyn Griffiths and Andrew Derksen (USDA-APHIS, CAPS; Miami, Florida) for assistance in collecting host material; and Jorge Peña (University of Florida; Homestead, Florida) and David Jenkins (USDA-ARS; Mayaguez, Puerto Rico) for helpful suggestions with the manuscript. This work was supported in part by the USDA-ARS National Plant Disease Recovery System, a NIFA Critical Issues Grant, and the Florida Avocado Administrative Committee. This report presents the results of research only; mention of a proprietary product does not constitute an endorsement by the USDA.

References Cited

BRENDEMUEHL, R. H. 1990. *Persea borbonia* (L.) Spreng. Redbay, pp. 503-506 *In* R. M. Burns and L. H. Honkala [eds.], Silvics of North America, vol. 2, Hardwoods. Agriculture Handbook 654. U.S. Government Printing Office. Washington, DC.

BROWN, C. L., AND KIRKMAN, L. K. 1990. Trees of Georgia and Adjacent States. Timber Press, Portland, Oregon.

BYERS, J. A. 1995. Host tree chemistry affecting colonization in bark beetles, pp. 154-213 *In* R. T. Cardé and W. J. Bell [eds.], Chemical Ecology of Insects 2. Chapman and Hall, New York, New York.

BYERS, J. A., ZHANG, Q. H., AND BIRGERSSON, G. 2000. Strategies of bark beetle, *Pityogenes bidentatus*, in an olfactory landscape. Naturwissenschaften 87: 503-507.

BYERS, J. A., ZHANG, Q. H., AND BIRGERSSON, G. 2004. Avoidance of non-host plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors. Naturwissenschaften 91: 215-219.

CISEH. 2010. Forestry images. Lindgren funnel trap. Center for Invasive Species and Ecosystem Health, USDA Forest Service, and International Society of Arboriculture. <http://www.forestryimages.org/browse/detail.cfm?imgnum=5426963>

CODER, K. D. 2006. Redbay (*Persea borbonia*): a tree of confusion. Outreach publication SFNR06-3. Warnell School of Forestry and Natural Resources, University of Georgia. <http://www.urbanforestrysouth.org/resources/library/redbay-persea-borbonia-a-tree-of-confusion/file_name>

CROOK, D. J., KHRIMIAN, A., FRANCESE, J. A., FRASER, I., POLAND, T. M., SAWYER, A. J., AND MASTRO, V. C. 2008. Development of a host-based semiochemical lure for trapping emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). Environ. Entomol. 37: 356-365.

EVANS, E. A., AND CRANE, J. H. 2008. Estimation of the replacement costs of commercial and backyard avocado trees in south Florida. Circular FE 825. University of Florida, Department of Food and Resource Economics. <http://edis.ifas.ufl.edu/pdffiles/FE/FE82500.pdf>

FDACS-DPI. 2010. Firewood movement rule (5B-65). Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Cooperative Agricultural Pest Survey Program. <http://www.freshfromflorida.com/pi/caps/firewood.html>

FDACS. 2011. Laurel wilt disease identified in Miami-Dade County. Press Release, 25 February 2011. Florida Department of Agriculture and Consumer Services. <http://www.freshfromflorida.com/press/2011/02252011.html>

FRAEDRICH, S. W., HARRINGTON, T. C., AND RABAGLIA, R. J. 2007. Laurel wilt: a new and devastating disease of redbay caused by a fungal symbiont of the exotic redbay ambrosia beetle. Newsl. Michigan Entomol. Soc. 52: 14-15.

FRAEDRICH, S. W., HARRINGTON, T. C., RABAGLIA, R. J., ULYSHEN, M. D., MAYFIELD III, A. E., HANULA, J. L., EICKWORT, J. M., AND MILLER, D. R. 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.

GERSHENZON, J., AND CROTEAU, R. 1991. Terpenoids, pp. 165-219 *In* G. A. Rosenthal and M. R. Berenbaum [eds.], Herbivores. Their Interactions with Secondary Plant Metabolites. The Chemical Participants. Academic Press, New York, New York.

HANULA, J. L., AND 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.

HANULA, J. L., MAYFIELD III, A. E., FRAEDRICH, S. W., AND RABAGLIA, R. J. 2008. Biology and host associations of the 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.

HARRINGTON, T. C., FRAEDRICH, S. W., AND AGHAYEVA, D. N. 2008. *Raffaelea lauricola*, a new ambrosia beetle symbiont and pathogen on the Lauraceae. Mycotaxon 104: 399-404.

HARRINGTON, T. C., AGHAYEVA, D. N., AND FRAEDRICH, S. W. 2010. New combinations of *Raffaelea, Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the redbay ambrosia beetle, *Xyleborus glabratus*. Mycotaxon 111: 337-361.

HEATH, R. R., AND MANUKIAN, A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. J. Chem. Ecol. 18: 1209-1226.

HEATH, R. R., MANUKIAN, A., EPSKY, N. D., SIVINSKI, J., CALKINS, C. O., AND LANDOLT, P. J. 1993. A bioassay system for collecting volatiles while simultaneously attracting tephritid fruit flies. J. Chem. Ecol. 19: 2395-2410.

HEATH, R. R., EPSKY, N. D., MIDGARDEN, D., AND KATSOYANNOS, B. I. 2004. Efficacy of 1,4-diaminobutane (putrescine) in a food-based synthetic attractant for capture of Mediterranean and Mexican fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 97: 1126-1131.

HSPD-9. 2009. Recovery Plan for Laurel Wilt on Redbay and other Forest Species Caused by *Raffaelea lauricola*, vector *Xyleborus glabratus*. National Plant Disease Recovery System. Homeland Security Presidential Directive Number 9. 27 pp. <http://www.ars.usda.gov/SP2UserFiles/Place/00000000/opmp/ForestLaurelWilt100107.pdf>

IBRAHIM, M. A., EGIGU, M. C., KASURINEN, A., YAHYA, A., AND HOLOPAINEN, J. K. 2010. Diversity of volatile organic compound emissions from flowering and vegetative branches of Yeheb, *Cordeauxia edulis* (Caesalpiniaceae), a threatened evergreen desert shrub. Flavour Fragr. J. 25: 83-92.

KENDRA, P. E., EPSKY, N. D., MONTGOMERY, W. S., AND HEATH, R. R. 2008. Response of *Anastrepha suspensa* (Diptera: Tephritidae) to terminal diamines in a food-based synthetic attractant. Environ. Entomol. 37: 1119-1125.

KENDRA, P. E., MONTGOMERY, W. S., NIOGRET, J., PEÑA, J. E., CAPINERA, J. L., BRAR, G., EPSKY, N. D., AND HEATH, R. R. 2011a. Attraction of the redbay ambrosia beetle, *Xyleborus glabratus*, to avocado, lychee, and essential oil lures. J. Chem. Ecol. 37: 932-942.

KENDRA, P. E., SANCHEZ, J. S., MONTGOMERY, W. S., OKINS, K. E., NIOGRET, J., PEÑA, J. E., EPSKY, N. D., AND HEATH, R. R. 2011b. Diversity of Scolytinae (Coleoptera: Curculionidae) attracted to avocado, lychee, and essential oil lures. Florida Entomol. 94: 123-130.

MAYFIELD III, A. E., PEÑA, J. E., CRANE, J. H., SMITH, J. A., BRANCH, C. L., OTTOSON, E. D., AND HUGHES, M. 2008. Ability of the redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) to bore into young avocado (Lauraceae) plants and transmit the laurel wilt pathogen (*Raffaelea* sp.). Florida Entomol. 91: 485-487.

MILLAR, J. G., ZHAO, C.H., LANIER, G. N., O'CALLAGHAN, D. P., GRIGGS, M., WEST, J. R., AND SILVERSTEIN, R. M. 1986. Components of moribund American elm trees as attractants to elm bark beetles, *Hyluropinus rufipes* and *Scolytus multistriatus.* J. Chem. Ecol. 12:583-608.

MILLER, D. R., AND RABAGLIA, R. J. 2009. Ethanol and (-)-α-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern U.S. J. Chem. Ecol. 35: 435-448.

MILLER, J. R., AND STRICKLER, K. L. 1984. Finding and accepting host plants, pp. 127-157 *In* W. J. Bell and R. R. Cardé [eds.], Chemical Ecology of Insects. Chapman and Hall, London.

NIOGRET, J., MONTGOMERY, W. S., KENDRA, P. E., HEATH, R. R., AND EPSKY, N. D. 2011. Attraction and electroantennogram responses of male Mediterranean fruit fly (Diptera: Tephritidae) to volatile chemicals from *Persea, Litchi*, and *Ficus* wood. J. Chem. Ecol. 37: 483-491.

PETRICE, T. R., HAACK, R. A., AND POLAND, T. M. 2004. Evaluation of three trap types and five lures for monitoring *Hylurgus ligniperda* (Coleoptera: Scolytidae) and other local scolytids in New York. Gt. Lakes Entomol. 37: 1-9.

PETTERSSON, E. M., SULLIVAN, B. T., ANDERSON, P., BERISFORD, C. W., AND BIRGERSSON, G. 2000. Odor perception in the bark beetle parasitoid *Roptrocerus xylophagorum* exposed to host associated volatiles. J. Chem. Ecol. 26: 2507-2525.

RABAGLIA, R. J. 2002. XVII. Scolytinae Latreille 1807, pp. 792-805 *In* R. H. Arnett, Jr., M. C. Thomas, P. E. Skelley, and J. H Frank [eds.], American Beetles, vol. 2, Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton, Florida.

RABAGLIA, R. J., DOLE, S. A., AND COGNATA, A. I. 2006. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. Ann. Entomol. Soc. Am. 99: 1034-1056.

SCOTT, J. A. 1986. The Butterflies of North America: A Natural History and Field Guide. Stanford University Press. Stanford, California.

SINGH, G., MARIMUTHU, P., DE HELUANI, C. S., AND CATALAN, C. A. N. 2007. Chemical constituents, antioxidative and antimicrobial activities of essential oil and oleoresin of tailed pepper (*Piper cubeba* L.). Int. J. Food Eng. 3: 1-22.

USDA-FS. 2011. Laurel wilt distribution. United States Department of Agriculture, Forest Service, Forest Health Protection, Southern Region. <http://www.fs.fed.us/r8/foresthealth/laurelwilt/dist_map.shtml>

USDA-NASS. 2011. Noncitrus fruits and nuts: 2010 preliminary summary. United States Department of Agriculture, National Agricultural Statistic Service. <http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-01-21-2011.pdf >

VISSER, J. H. 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 31: 121-144.

YONG, Y., AND WENYI, T. 2005. Headspace solid phase micro-extraction followed by gas chromatography - mass spectrometry analysis of the volatile components in seeds of *Cinnamomum camphora*. Am. J. Biochem. Biotech. 1(3): 174-176.

ZHU, F., AND GUO, Y. 2010. Analysis of the volatile components in the leaves of *Cinnamomum camphora* by static headspace gas chromatography - mass spectrometry combined with accurate weight measurement. Chinese J. Chem. 28, 8: 1451-1456.